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PB-300 380

The National Environmental Specimen Bank Research Program for Sampling, Storage and Analysis

(U.S.) Health Effects Research Lab, Research Triangle Park, NC

May 79

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

EPA-600/1-79-017
May 1979

Research and Development

PB 300380

EPA

The National Environmental Specimen Bank Research Program for Sampling, Storage, and Analysis

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-79-017	2.
3. RECIPIENT'S ACCESSION NO. PB300389	
4. TITLE AND SUBTITLE THE NATIONAL ENVIRONMENTAL SPECIMEN BANK RESEARCH PROGRAM FOR SAMPLING, STORAGE, AND ANALYSIS	5. REPORT DATE May 1979
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) T.E. Gillis, H.L. Rook, R.A. Durst, Center for Ana. Chemistry, and National Bureau of Standards Washington,DC	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Clinical Studies Division Health Effects Research Laboratory Research Triangle Park, N.C. 27711	10. PROGRAM ELEMENT NO. 1HE775
	11. CONTRACT/GRANT NO. EPA-78-D-X0105-1
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory HERL, RTP Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, N.C. 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This work was performed under a joint NBS/EPA research program to develop state-of-the-art protocols for sampling, storage, and analysis of biological and environmental-type matrices. This report is a compilation of research papers and/or efforts describing developed or adopted procedures for retrospective analysis of biological and environmental samples. Preliminary protocols for sampling, sample handling, and sample storage are given for human liver autopsy tissue in addition to methods for the accurate measurement of selected toxic elements in biological and environmental materials. Analytical methods employed were neutron activation analysis (NAA), polarography, and isotope dilution spark source mass spectrometry (IDSSMS).

This report is submitted in partial fulfillment of EPA Interagency agreement EPA-78-D-X0105 by the National Bureau of Standards for the 1978 contract year.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
toxic elements polarography environmental samples lung tissue		06,c,e,f,t 07,c

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 70
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE A04 - A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/1-79-017
May 1979

THE NATIONAL ENVIRONMENTAL SPECIMEN BANK RESEARCH PROGRAM FOR SAMPLING, STORAGE, AND ANALYSIS

by

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report documents one aspect of an International effort, supported by EPA, to provide a comprehensive environmental monitoring program to assess the relative risk of environmental hazard to the health and well-being of our population and to aid in the improvement of our environmental quality. This program, the National Environmental Specimen Bank, will serve as an environmental warning system by providing real time chemical analysis of collected specimens. In addition, this system would permit the use of tomorrow's more sensitive and more specific methods of chemical analysis on stored samples. The advantages of such a program will permit us to assess the effectiveness of our present environmental control techniques by monitoring pollutant trends, as well as establishing environmental baseline levels of new pollutants or pollutants of current concern not previously investigated.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

This work was performed under a joint NBS/EPA research program to develop state-of-the-art protocols for sampling, storage, and analysis of biological and environmental-type matrices. This report is a compilation of research papers and/or efforts describing developed or adopted procedures for retrospective analysis of biological and environmental samples. Preliminary protocols for sampling, sample handling, and sample storage are given for human liver autopsy tissue in addition to methods for the accurate measurement of selected toxic elements in biological and environmental materials. Analytical methods employed were neutron activation analysis (NAA), polarography, and isotope dilution spark source mass spectrometry (IDSSMS).

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ACKNOWLEDGMENTS

The authors of this report wish to acknowledge their appreciation to the Environmental Protection Agency for its funding. We especially want to thank the Project Officer, Dr. George M. Goldstein, Chief of the Clinical Pathology Branch for his consultations and program guidance.

SECTION 1

DEVELOPMENT OF A PRELIMINARY PROTOCOL FOR SAMPLING, SAMPLE HANDLING, AND LONG-TERM STORAGE OF HUMAN LIVER

by

E. June Maienthal

INTRODUCTION

In response to the increasing concern with environmental pollution, the National Bureau of Standards (NBS) in collaboration with the Environmental Protection Agency (EPA) has developed a preliminary protocol for sampling, sample handling and long-term storage of human livers, which are to be monitored for trace element and trace organic compositions for the Pilot National Environmental Specimen Bank which will be located at NBS. The criteria for arriving at these guidelines will be discussed, and the preliminary guidelines up to the stage of subsampling will be given.

Considerations

From an earlier literature survey (1), it was obvious that much of the work concerning environmental sampling, sample handling, and long-term storage is of questionable value owing partially to sources of contamination from various steps in the sampling and sample processing or to losses of some of the trace constituents through adsorption, volatility, etc. It was also obvious from a survey of existing environmental collections that few of the collections surveyed would be of sufficient value for environmental trace inorganic and organic determinations (2). It was necessary, therefore, prior to the establishment of the Pilot National Environmental Specimen Bank, to set up a valid working protocol.

The rationale and plans for the National Environmental Specimen Bank (NESB) have been described by Goldstein, where he states ". . . regardless of the outcome of the NESB, the methodology protocol development would provide the scientific community with state-of-the-art standardized protocols for sample collections in a variety of ecologically important materials. The cost benefit of this alone is incalculable." (3).

The protocol to be described was arrived at as the result of work conducted at NBS, a past literature survey (1), and additional literature investigated and consultations with other workers in the field, including chemists, biochemists, pathologists, cryobiologists, physicians, and equipment suppliers. The necessity of obtaining and storing a sample valid for both trace organic and inorganic constituents requires very rigid control of the sampling materials and methods, shipping methods, storage containers and storage conditions.

Although shipping and storage are the last items in the protocol, they will be discussed first in order to explain the reasons for certain other steps of the procedure. It was generally concluded that it would be necessary to freeze the specimen as soon as possible after sampling for long-term banking. Frozen storage is usually done at about -20 °C (approximate home freezer temperature), -80 °C (approximate dry ice temperature), or at -196 °C (liquid nitrogen temperature). At -20 °C, however, samples often may not be frozen, and at -80 °C, molecular or enzymatic activity may still occur (4,5). Although this might not affect the stability of the inorganic constituents, some speciation and organic changes could occur. Some chemical changes are reported to occur as low as -130 °C (5), and some physical changes such as devitrification are reported to take place at temperatures substantially lower than -130 °C (6). Meryman had earlier reported that no biochemical activity should take place below -130 °C (7), but states that on the basis of Dowell and Renfret's work (6), further study of this activity at low temperatures should be investigated (7a). Mazur has reported that at -196 °C, no aqueous reaction occurs and that a tissue sample should be preserved, unchanged indefinitely at that temperature (8). Cravalho also states that to arrest biochemical and physical processes, the lower the storage temperature the better, and that there is no lower temperature limit for storage of biomaterials (8a). It would appear therefore, that liquid nitrogen would be an ideal storage as well as shipping medium, having the additional advantage of being relatively inexpensive and readily available. Laessig, et al. describe some of the earlier uses of liquid nitrogen for storing and shipping biological samples in tanks by air freight with no difficulty (9). These tanks were used by Laessig, et al. for epidemiological serum studies in remote field areas and were found adaptable under the most severe circumstances. The tanks are commercially available from several manufacturers and are being used routinely for tissue shipping and storage by groups such as the Tissue Bank of the Naval Medical Research Institute. The use of dry ice has not been as satisfactory for shipping, in part because of frequent failures and thawing of samples, and also because of possible pH changes of the sample owing to diffusion of carbon dioxide into the container (4). Because of cell rupture and tissue leakage during thawing, a sample cannot be salvaged for many analytical purposes by refreezing once it has been thawed. Omang and Vellar have described the concentration gradients occurring in biological samples during freezing and thawing (10).

The choice of donor specimen must also be under some restrictions. For the pilot study and to check the preliminary protocol, normal livers which show no erratic concentrations of the constituents to be determined are desired. For this reason, certain donors are to be excluded, such as those: having liver weights less than about 1000 grams; stored at temperatures above 4 °C; previously frozen; deceased more than 24 hours; a history of alcoholism, gross sepsis, viral hepatitis, tuberculosis, cirrhosis, liver carcinoma, chronic circulatory failure or congestion, chemical or drug overdose or exposure; or previously embalmed.

Ideally the sample should be taken immediately after donor death since significant enzymatic changes occur with time (11), but at present this would not be generally possible; so on a practical basis an upper limit of 24 hours has been set.

Ideally, the autopsy should be performed in a laminar-flow clean room atmosphere, because the chances of contamination from the typical autopsy room environment are very great. Again, this is not at present a practicality; hence, as few manipulations as possible are to be done prior to sealing the sample in the cleaned container.

Most autopsies must be performed in a sterile atmosphere requiring the use of sterile surgeon's gloves which are typically latex, powdered both inside and out. This type of glove would be prone to introduce trace element contaminations, such as zinc from the latex itself, and other trace elements from the powder. A producer of sterile, clean-room packaged, talc-dust free, polyvinyl chloride gloves has been located, and these gloves have been found acceptable for use at the autopsy facilities so far contacted. It must be remembered, however, that there may be a chance of trace organic contaminations of the liver surface from the plastic or plasticizers used in the PVC, as will be discussed later. This organic contamination could occur with latex gloves also. For the first samples taken, relatively standard surgical instruments are to be used — unrusted, cleaned steel for the liver removal, and an unrusted, cleaned, hardened carbon steel knife for slicing the left lobe into two equal sections. It must be remembered that possible surface contamination from the knives may occur, as discussed previously (1), particularly as shown by Versieck, Speecke and coworkers (12,13), and by Maletskos, et al. (13a). A number of other types of less-contaminating implement materials are being considered and will be discussed later.

The liver section should be rinsed with pure distilled water to remove gross external contamination, put into its container, sealed, and immediately frozen in liquid nitrogen.

One problem arising from freezing a section of liver complete with its blood and extracellular fluid is that the amount of fluid contained can vary greatly in a normal human being, depending on time and a variety of physiological factors, thus causing an apparent difference in compositions from sample to sample because of varying amounts of fluid present at time of death. The fluid can differ markedly in composition from that of the tissue. This has been shown by LeBaron and coworkers by analysis of human kidney tissues and fluids (13b). Iyengar and Kasperek have used a very promising approach which will be investigated here and possibly incorporated into the protocol (13c). They rinse the liver sections three times, squeezing the sections gently between clean polyethylene sheets (Teflon would probably be used here) in between rinsing processes, thereby eliminating most of the blood. The sections were then frozen in liquid nitrogen and gently pressed between two Perspex plates. This results in the fracture into small pieces which could be picked out with plastic forceps, separating the remaining blood vessels and homogenizing the rest by the brittle fracture technique using a special Teflon vessel and a Teflon ball with a metal core (14).

A very important item of concern is the choice of container and this is discussed in more detail in another section of this report. This also was discussed at length previously (1) and in the Proceedings of the 7th Materials Research Symposium (14a). It would be desirable to have one type of sample container suitable for both organic and inorganic constituents. Although some plastics can be cleaned sufficiently for trace element analysis (1,15), the contamination for organic constituents and migration of plasticizers has been

often shown (1,16,17,18). Also, most of the plastics will not withstand liquid nitrogen temperatures without breaking. One lot of polypropylene cryogenic tubes also showed the presence of sodium, aluminum, chloride, and titanium (18a). Properly cleaned Pyrex containers are suitable for organic constituents, but are notoriously bad for trace elements through contamination and/or adsorption (1,19). The latter reference also found significant zinc contamination from polypropylene containers (19).

The polyfluorinated hydrocarbons such as Teflon, FEP or PFA, after appropriate cleaning appear to be the most suitable for both organic and inorganic constituents. They will also withstand liquid nitrogen temperatures if not subjected to too severe physical stresses.

A preliminary experiment was done at NBS freezing pork liver in a heat-sealed 5-mil Teflon FEP bag in liquid nitrogen with promising results (19a). The bags are evacuated immediately prior to sealing to increase the sample stability (20,21). The packaged sample should be put in a forming cylinder prior to freezing in liquid nitrogen in order to obtain a more compact, easily handled package for shipping and storing.

The liver sections are weighed separately in order that weight loss or gain can be checked after they are received at NBS and periodically during storage. One problem with Teflon is that it is porous to gases, and the samples may be subject to moisture loss or gain. If this should prove to be a problem, a vapor barrier such as aluminized Mylar or glass may be required in the protocol.

The liquid nitrogen shipping containers can also be used as storage containers prior to shipping the livers to NBS. These containers have an absorbent around the inner walls, so that during shipping only vapor-phase nitrogen need be present. The type which will probably be used for the first experiments has a static holding time of 11 days after the absorbent is saturated.

The two halves of the left liver lobe are to be sent back to NBS in order that different subsampling and storage techniques may be investigated. One section will be stored untouched in the event that the subsampling procedures (as yet unfinalized) should in some way alter or contaminate the other section. It must also be emphasized that the sampling protocol is preliminary and will necessarily change as unforeseen problems are encountered and improved methods arise. It is now thought that the subsampled section will be stored in approximately 8 mL PFA bottles (which may also require an outside vapor barrier). It is possible that glass might be used for the samples for the organic determinations.

A major problem remaining is how to subsample and homogenize the liver section without contaminating it. Various methods have been suggested, including Waring blenders, meat grinders, ultrasonic tissue homogenizers, food processors, cryogenic grinding and brittle fracture. Some preliminary studies have been made at NBS on subsampling, homogenization, analysis by neutron activation, freezing, storing, and reanalysis of beef liver (21a,21b). A number of types of sampling tools have been used which seem to offer much less chance of contamination than the steel knives mentioned earlier. Iyengar and Kasperek used a specially prepared Suprasil-quality-quartz knife for slicing

the liver, and used the brittle fracture technique, as mentioned earlier, at liquid nitrogen temperatures utilizing a specially prepared Teflon container and Teflon ball with a metal core (13c). Lievens, et al. also used an ultra-clean spec-pure quartz tube to obtain liver subsamples (22). Thompson and Bankston studied contamination arising from grinding and sieving devices made from different types of materials (23). They found that a boron carbide mortar introduced little or no contamination (except for boron) and that stainless steel or brass sieves introduced appreciable levels of cobalt, copper, iron, manganese, nickel, lead, tin, and zinc. Maletskos and coworkers used a stainless steel knife to cut a frozen segment of tissue, then a special high purity boron nitride knife to shave the surfaces which had been in contact with the stainless steel (13a). Folsom developed several different types of plastic sampling tools for frozen tissue sampling (24). These, however, might not be suitable for trace organic samples. Another interesting sampling possibility involves the use of the laser. Hislop and Parker used a carbon dioxide laser for cutting bone with no trace element contamination (25). Auth, Doty, and coworkers have developed a "laser blade" or "laser scalpel" using high power argon laser radiation transported via a low-loss optical fiber into a sharp transparent quartz knife (26). This device has proved very useful for operations in which massive bleeding is a problem. This device has the effect of cauterizing the incision and sealing the blood vessels. Of course for the trace constituent analysis the several millimeters of cauterized tissue would need to be removed by an implement which would be noncontaminating for the constituents of interest for the particular determination.

A number of additional references concerned with problems involved in trace analysis of tissue or biological matrices are listed (27-44).

It must be emphasized again that the attached protocol is preliminary and will change as improved methods are developed.

LIVER SAMPLING PROTOCOL

I. DEMOGRAPHIC DATA

a. Record autopsy identification number and NBS number on label provided and attach to sample bag (Note 1).

b. Collect and include donor information with shipment and send a copy under separate cover. Donor information should contain as a minimum: the autopsy identification number; the NBS number; date and time of death; date and time of autopsy; weight of whole liver and separate left lobe sections; donor height; weight; age; sex; and ethnic group (Note 2).

II. SAMPLE HANDLING

a. Sterile, cleaned PVC gloves will be provided and should be used for liver removal and handling. Extreme precaution must be taken throughout the autopsy procedure to reduce the risk of contaminating the liver sample. Contamination may result during the autopsy from the donor, the individual(s) performing the autopsy, the atmosphere and/or the surgical instruments.

III. SAMPLING

a. The liver is identified and excised with instruments supplied by NBS. The liver should be removed as early as is feasible after death (but not later than 24 hours) and as close to the beginning of the autopsy as normal routine permits (Note 3).

The liver will <u>not</u> at any time during the autopsy procedure be placed on any surface other than cleaned Teflon sheets provided by NBS.

b. The liver is placed on an inclined surface (to provide rapid drainage) covered with a Teflon sheet. Washing is accomplished by pouring approximately 250 mL of distilled water (Note 4) over the liver surface. The liver is then turned over and the washing procedure repeated.

c. Excess water is allowed to drain off the liver prior to weighing on another Teflon sheet.

d. Left lobe is dissected with the knife provided by NBS.

e. Left lobe is cut into equal halves through an axis of symmetry with the knife provided.

f. A section is taken from a cut surface of the left lobe (with the knife supplied) for the preparation of a histological slide. A slide and copy of the pathological findings is sent under separate cover as per Ib above.

g. Each liver section is immediately placed in separate Teflon bags provided by NBS and weighed separately on Teflon sheet as in IIIc.

h. Double heat seal each bag (with the twin heat sealer supplied by NBS) to within about 1 inch of one side. Insert a precleaned Teflon tube, attached to a hand vacuum pump (both supplied by NBS), into the unsealed corner of the bag. Evacuate each bag and double heat seal across the remaining open corner while maintaining the vacuum.

i. The sealed Teflon bags are then placed in the Teflon cylinders, provided by NBS, and frozen by immersion for at least 15 minutes in liquid nitrogen contained in a plastic Dewar supplied by NBS (Note 5).

j. Remove the Teflon bag from the cylinder and inspect the bag for obvious damage. If loss of sample integrity is encountered, both liver sections should be discarded.

k. Prefill the shipping container with liquid nitrogen so that there is at least 4 inches of standing liquid. This may be estimated by using a dipstick. The liquid nitrogen level must be maintained (Note 6).

l. Transfer the Teflon-packaged frozen liver sections to the shipping container. Check and maintain the liquid nitrogen level as directed in IIIk above until ready for shipment.

m. Store liver specimens no longer than 10 days or until 4 lobes (8 sections) have been collected.

n. Draw off excess liquid nitrogen, cap securely, and ship to the National Bureau of Standards by a carrier (to be designated later). Include with the shipment a copy of the information listed in Ia and Ib, as well as the Restricted Article Statement required by the CAB. Copies of information from Ia and Ib, along with the histological slide should also be sent under separate cover (mentioned in Ib, IIIf above) (Note 7).

NOTES FOR PROTOCOL

1. Polyester tape, labels, and dispenser are to be supplied by NBS. Labels are to be affixed to the sample bag by wrapping tape completely around bag and overlapping ends one-half inch.

2. At a later date in this protocol, additional donor information will be sought for a specified number of samples. This will include: smoking and drinking habits, socioeconomic status, residential environment, and employment history.

3. Liver samples are to be excluded if donors have: a total liver weight of less than 1000 grams (35 oz); livers which have been ruptured or punctured; been stored at temperatures above 4 °C, or have been previously frozen; had a history of alcoholism, drug addiction, or long-term drug therapy; had gross sepsis, viral hepatitis, tuberculosis, cirrhosis, liver carcinoma; had a history of chronic circulatory failure or congestion; died due to chemical overdose or exposure; or embalmed donors.

4. Water used in washing the liver will be provided by NBS or obtained from a local source that has been previously analyzed by NBS and shown to be of acceptable quality.

5. Precautions should be observed when working with liquid nitrogen. Liquid nitrogen should not be stored in sealed containers. When transporting by elevator, personnel should not accompany Dewar because of the possibility of elevator and/or Dewar failure. Personnel handling liquid nitrogen are cautioned to wear boots, cuffless trousers, non-absorbent apron, loose, insulating gloves and face shields.

6. The cryogenic shipping container weighs 27 kg (59 lbs) empty. With the absorbent filled with liquid nitrogen, the weight increases to 38 kg (84 lbs). The container requires approximately 14 liters to fill the absorbent. The static evaporation rate is approximately 1.2 liters/day.

7. A Government Bill of Lading (GBL) will be supplied to cover shipping expenses. Forms for the Restricted Article Statements will be provided by NBS. These forms must be included with each shipment.

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SECTION 2

RESEARCH ON FREEZER STORAGE

by

T. E. Gills, S. Harrison, and H. L. Rook

An important part of the NESB research program has been a study and evaluation of freezer storage for long-term storage of tissues and/or other biologically active samples. The effects of microbiological action on trace constituents concentrations and distributions are well documented. Freezing has long been applied as a technique for storage, however no study has been previously made to document the reliability with respect to inorganic ions of this method of storage for more than a short period of time. Recently, the analysis and subsequent reanalysis of a selected animal tissue (bovine liver) that had been stored frozen at -80 °C for a period of one year gave important information on the effects of freezer storage for select elements, namely iron, copper, zinc, molybdenum, selenium, cobalt, and arsenic. The original organ had been subset sampled using stainless steel scalpel blades and the remaining portion homogenized using a commercial food blender. The homogenate was poured into six precleaned polypropylene ice cube trays which were sealed in 1 mil thick polyethylene film and stored in a chest-type -80 °C freezer. Analyses were performed on the subsets and homogenates to provide initial trace elemental concentrations and to verify contamination-free homogenization of the sample. For the one year reanalysis, one tissue cube was removed from each of the six trays and the trays resealed for future studies. The selected tissue cubes were freeze-dried and analyzed using the same analytical procedures utilized in the initial analysis. A comparison of the results of the reanalysis to those originally obtained is given in Table I. For the elements determined, the capability to procure a tissue and store it frozen for one year for retrospective analysis has been demonstrated. In addition, results indicate that subset sampling can be a viable sampling strategy when applied to relatively homogenous tissues such as liver, etc. Important elements such as chromium, cadmium, iodine, lead, etc., though not analyzed in this set of samples, are currently being evaluated for their stability in tissues during freezer storage.

The sampling implements used in this work are not suggested for sampling or homogenization but were utilized to provide a base for evaluating contamination with respect to elements such as chromium. Work is currently being done to assess contamination from sampling implements and their effects upon analytical uncertainty of real data.

Table I
Analyses of a Stored Bovine Liver
Storage and Analysis Protocol Tests
(Concentrations Reported on Dry Wt. Basis)
µg/g

Sample Type	No. of Samples	Fe	Cu	Zn	Mo	Se	Co	As (ng/g)	Analysis Procedure Used
I. Subset Samples (+)	18	76±7	68±10	39±3	.89±.12	.29±.05	.077±0.006	4±1	RNAA
II. Homogenate (*)	7	79±4	69± 5	40±2	.76±.04	.30±.05	.077±0.005	6±2	RNAA
III. Homogenate (+)	5	74±6	69± 3	39±2	.80±.05	.30±.03	.073±0.003	6±2	RNAA
IV. Subset Samples (+)	6	75±4	--	39±1	--	.29±.02	.075±0.002	--	INAA
AVERAGE	36	76±2	69± 1	39±1	.82±.07	.29±.01	.075±0.002	5±1	
Error: Standard Deviations of Means									
III. Reanalysis after 1 yr (+,*)	6	69±7	70± 2	38±1	.82±.08	.27±.05	.076±0.003	4±1	RNAA

+ Fresh or Frozen Tissue

* Freeze-dried

SECTION 3

CONTAINER MATERIALS FOR THE PRESERVATION OF TRACE SUBSTANCES IN ENVIRONMENTAL MATERIALS

by

Richard A. Durst

INTRODUCTION

The success of the National Environmental Specimen Bank will be determined in large part by the ability to preserve the integrity of the trace substances in samples during long-term storage. It is widely recognized that the storage of environmental materials is subject to a variety of uncertainties when one is considering the identity and levels of trace elements and organic compounds.

Changes in the forms and concentrations of the numerous environmentally important substances in specimens stored for extended periods may occur in several ways. Processes such as surface adsorption and sample degradation may reduce the concentrations of various components and/or produce species which may not have been present in the original sample. On the other hand, contamination from the container and evaporation of specimen fluids could lead to apparent increases in trace substance concentrations or losses in the case of volatile trace components. In addition, superimposed on these processes are the factors which will affect their rates, such as, container material, contact time and area, storage temperature, pH, and initial species concentration. All of these factors are important considerations in evaluating the suitability of various containers for long-term storage.

There have been numerous studies performed in recent years directed toward the identification of suitable container materials and optimum storage conditions, but much of this work is contradictory (1). In many cases, the analytical data were invalidated because of problems not associated with the storage but with other links in the analytical chain such as sampling and procedural contamination.

Container Materials

While certain container materials can be eliminated *a priori*, it is usually necessary to consider the container composition vis-a-vis the type of sample and/or the components of interest in the sample. Accordingly, it is unlikely that samples intended for trace organic analysis would be stored in plastic containers or acidified water samples in glass containers if trace elements were to be determined. While these are obvious examples of

incompatible sample/container combinations, even these may be acceptable under certain storage conditions and for special requirements.

The National Environmental Specimen Bank is somewhat unique in that both trace elements and trace organics are of environmental concern and a choice must be made as to storing the samples in a single container material suitable for both types of components or in two different container materials optimized for the components of interest. In the former case, the choices are much more limited and compromises must be made, whereas in the latter, the increased complexity of separate sampling, sample handling and storage procedures may be excessive. In the EPA/NBS Environmental Pilot Bank, this is one of the questions to be answered by careful evaluation of a variety of container materials under different cleaning procedures and storage conditions.

At NBS, most of the research to date has been concerned with trace element contamination and losses, while trace organic problems have largely been avoided by the use of carefully cleaned glass containers and immediate freezing of the samples. In a recent study (2), twelve different plastics were examined by gravimetry, isotope dilution mass spectrometry, and neutron activation analysis in order to evaluate the rate of water loss, the levels of impurities present in the plastics, and the quantities of trace elements leached from the plastics during acid cleaning.

The annual rate of water loss, based on weighings at 17 and 66 days, are given in Table 1. The polypropylene, Teflon, and conventional polyethylene have water loss rates compatible with long-term storage of aqueous samples, whereas the other plastics may be adequate for shorter storage periods (2).

TABLE 1. ANNUAL RATE OF WATER LOSS FROM PLASTIC CONTAINERS

Material	% Loss/year*
Polypropylene	0.04
Teflon	0.05
Conventional polyethylene	0.1
Polyvinyl chloride	0.5
Polymethylpentane	1
Polycarbonate	2

*Average values based on weight loss measured at 17 and 66 days.

Since the closure may have been responsible for a significant portion of the water loss, these values should not be construed as permeability data but merely typical losses from these types of containers. These losses could be reduced in practice by sealing the container in a vapor barrier or storage at an ambient relative humidity comparable to that of the aqueous samples.

The concentrations of trace elements contained in the plastics were determined by neutron activation analysis (2). The ten plastics studied showed

striking differences in trace element composition as illustrated in Table 2 for several of the elements determined.

Table 2. Trace elements in plastics determined by Neutron Activation Analysis (Concentrations given in ng/g)

Element	CPE	LPE	PP	PMP	PS	PC	TFE	FEP
Na	$1x10^3$	$15x10^3$	$5x10^3$	200	$2x10^3$	$3x10^3$	160	400
Al	500	$3x10^4$	$6x10^4$	$6x10^3$	500	$3x10^3$	230	200
Ti		$5x10^3$	$6x10^4$	$5x10^3$	$1x10^3$			
Mn		10	20	10	20			60
Co			40			6		
Zn		$5x10^5$		$3x10^4$				
Br	>20	800	>5	>2	>1	$3x10^4$	>2	
Sb	5	200	600					
Au			0.1	0.6	0.04	0.03	0.4	

CPE = conventional polyethylene; LPE = linear polyethylene;
PP = polypropylene; PMP = polymethylpentane; PS = polystyrene;
PC = polycarbonate; TFE and FEP = types of Teflon.

In agreement with the results of other workers, the purest materials appear to be Teflon, PS, and CPE. Comparison of these data with those obtained in leaching experiments indicate that the bulk of most trace element impurities present is distributed throughout the matrix, rather than concentrated at the surface.

The acid-leaching experiments were performed on four types of plastic using two acids prepared from ultra-pure reagents. In this study (2), the plastic bottles were first rinsed with distilled water to remove any surface contamination and then filled with a 1+1 mixture of ultra-pure HNO_3 or ultra-pure HCl and ultra-pure water (3). The leaching process was allowed to proceed for one week at room temperature (80 °C for the FEP bottle) before the analyses were performed. The results obtained by isotope dilution mass spectrometry are given in Table 3 for a selected group of the impurity elements measured.

The results of these experiments indicate that HNO_3 and HCl leach various elements with different efficiencies, and it is recommended that both acids, in sequence, be used for cleaning these containers (2). After cleaning, the Teflon and CPE bottles have been found to be the least contaminating. Based on experience at NBS and reports of other studies, a recommended method for cleaning plastic containers has been proposed (2):

1. Fill container with 1+1 analytical reagent grade HCl.

2. Allow to stand for one week at room temperature (80 °C for Teflon).

3. Empty and rinse with distilled water.

4. Fill with 1+1 analytical reagent grade HNO_3.

5. Repeat steps 2 and 3.

6. Fill with the purest available distilled water.

7. Allow to stand several weeks or until needed, changing water periodically to ensure continued cleaning.

8. Rinse with purest water and allow to dry in a particle- and fume-free environment.

Table 3. Impurities Leached from Plastic Containers (ng/cm^2)

Element	FEP		LPE		CPE		PC	
	HNO_3	HCl	HNO_3	HCl	HNO_3	HCl	HNO_3	HCl
Pb	2	2	2	0.6	0.7	18	0.3	10
Al	6	4	1	4	1	10	5	3
Na	6	2	10	6	8	42	3	8
Sn	1	1	1	<1	<0.8	<0.8	0.2	13
Cd	0.4	0.6	0.2	0.2	0.2	0.2	0.3	<8
Se	0.2	0.8	0.4	0.4	3	<0.3	0.5	<0.5
Zn	4	4	8	9	2	1	0.8	-
Cu	2	6	0.4	1	2	0.7	0.8	<6
Ni	2	0.8	1.6	0.8	0.5	0.3	0.7	0.3
Fe	20	16	3	1	3	1	3	<49
Cr	0.8	4	0.2	0.8	0.8	0.3	0.3	<5
Mg	8	1	0.6	0.4	0.7	0.7	2	0.8

Values which are below 2 ng/cm^2 or prefixed by < are upper limits.

For the storage of biological tissues and fluids, rapid freeze drying immediately after sampling has been recommended but suffers from the disadvantage that some volatile components may be lost (1). A more viable approach would appear to be the immediate freezing of the sample or sub-samples to the lowest conveniently attainable temperature. This approach serves two purposes: 1) it reduces or stops both chemical and biological processes which could result in sample changes, and 2) it reduces the mobility of sample and container components thereby lessening the possibility of contamination and/or losses due to adsorption or volatility. However, because of concentration gradients which may be produced during freeze-thaw cycles and physical changes, e.g., cell destruction, caused by freezing, it is highly recommended that all sub-sampling sites usually precludes this procedure because of the probability

of sample contamination during the sub-sampling. Instead, the sample, which has been frozen as rapidly as possible to avoid component fractionation, is sub-sampled while still in the frozen state at a location which has a clean facility.

When the sample is frozen, especially at cryogenic temperatures, the reduced interaction between the sample and container permits greater flexibility in the choice of container material. However, even under these conditions, it is inadvisable to store samples intended for trace organic analysis in any type of plastic container which incorporates additives such as plasticizers, organometallic or other stabilizer antioxidants, colorants, or any other components which may be leached or volatilized from the plastic. Teflon is the only plastic which has been found suitable for trace organic storage at cryogenic temperatures, and which, after proper cleaning, is also suitable for the storage of samples intended for trace element analysis.

Glass (quartz or Pyrex), with Teflon (or aluminum foil) lined caps, is also suitable for trace organic samples and may be satisfactory for frozen trace element samples. At the present time, the key to long-term storage appears to be storage at cryogenic temperatures to reduce reactions and interactions to a minimum. Under these conditions, both glass and Teflon appear to be suitable storage container materials.

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SECTION 4

DESIGN AND CONSTRUCTION OF THE PILOT BANK FACILITY

by

H. L. Rook, T. E. Gills, J. Koskoris, R. A. Durst

A final proposal and design for the construction of the Pilot Bank low-temperature storage facility has been made. The Pilot Bank facility will have the capacity to house 20 replicate samples for each of the approximately 4000 input specimens planned for the five-year storage period. These will be derived from four classes: human tissue, aquatic tissue, food grain, and air accumulator. Nominal sample size will be five-to-ten grams per replicate sample. Samples will be stored at approximately -196 °C liquid nitrogen temperature, -80 °C, -25 °C, and freeze dried material stored at room temperature. The multiple storage conditions for the same sample will allow direct evaluation of the candidate storage procedures. Protocols for sampling and sample handling as discussed earlier in this report will be followed.

The pilot facility will be made up of an existing NBS clean room which will be upgraded to a Class-100 clean lab, and a new Class-100 storage area which will be constructed adjacent to the existing clean room (see figure 1). The existing clean room will be modified to provide ultra-clean preparation laboratory conditions equivalent to a trace constituent analytical laboratory. Samples will be protected with ultra-clean air (99.97% efficient on all particles 0.3 micrometer and larger). Personnel will be protected by performing all operations with potentially pathogenic samples (liver) in a biological clean work station.

The air flow pattern and work function will be designed to eliminate or minimize contamination caused by work functions. The preparation laboratory will be divided into two principal work areas by means of a central dual air return wall. A conventional fume hood and clean air module providing vertical-flow, Class-100 air will be located in the inorganic analysis section. A biohazard enclosure and clean air module providing Class-100, charcoal-filtered air will be located in the organic analysis section of the laboratory. False walls will also be located at the ends of this laboratory to provide additional air return to the clean air modules containing HEPA filters.

The Class-100 storage area will consist of a room approximately 3.7 m by 7.3 m (12' x 24') connected to the existing clean lab via an air lock. The cleanliness of this storage area will have a particle count of fewer than 100

particles 0.3 micrometer and larger per cubic foot. Freezers will be located in this area for the storage of specimens in liquid nitrogen freezers (approximately -196 °C) and compressor-type freezers at -25 and -80 °C. Racks will also be provided for freeze-dried samples. In order to avoid the possibility of build up of potentially harmful levels of nitrogen which could lead to oxygen starvation, provision will be made to vent and replace sufficient storage room air to ensure safe oxygen levels. In addition, the freezer compressors will be hermetically sealed in order to minimize potential contamination from wear metals and/or organic vapors.

It is anticipated that this laboratory/storage facility will be completed in mid-summer of 1979. Human liver specimens will be acquired immediately upon completion of this facility.

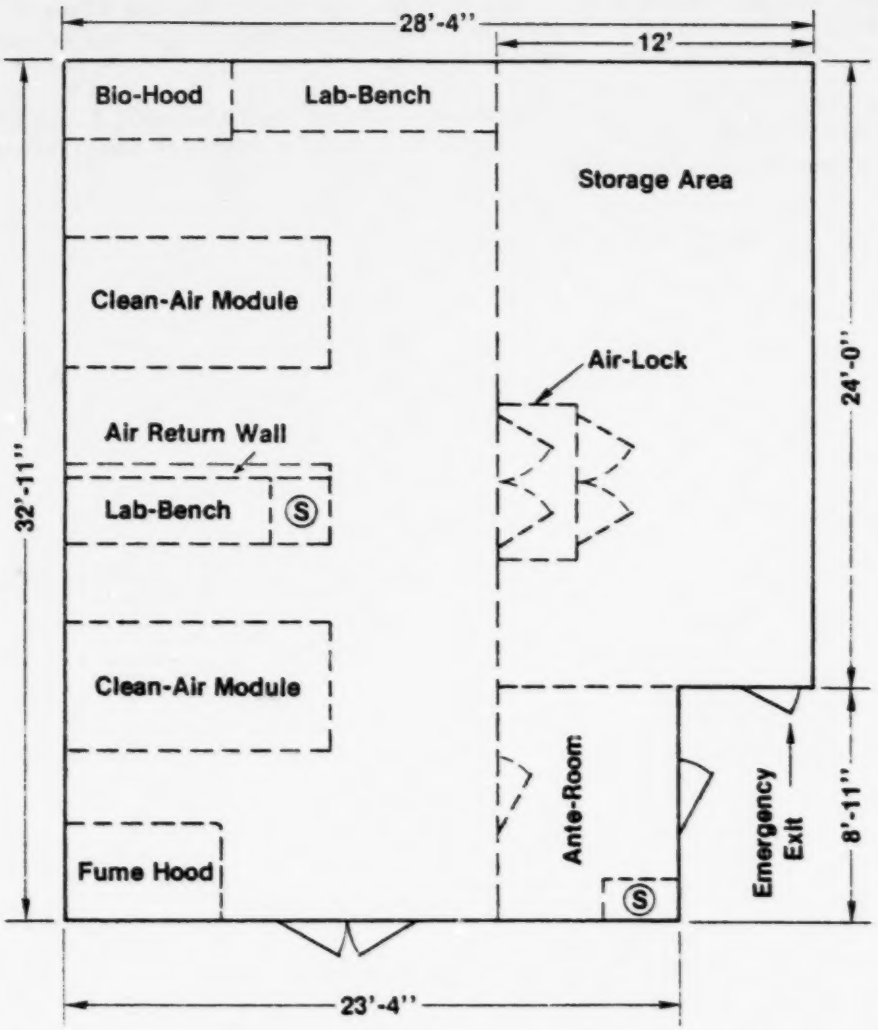

Figure 1. Specimen Bank Laboratory/Storage Facility

SECTION 5

ORGANIC MERCURY IN TISSUES

by

R. L. Zeisler, T. E. Gills

Increased interest of ecologists and analysts has been focused on the ratio of organic mercury to total mercury content of biological and environmental samples. The determination of the total mercury content of these matrices using Neutron Activation Analysis (NAA) procedures has been extensively investigated in the past years, and some Radiochemical Neutron Activation Analysis (RNAA) methods are considered fully reliable for this purpose. So far, the determination of organic mercury has been carried out by use of other methods, e.g. gas-chromatography. Thus, two samples have to be analyzed by different methods to yield the desired information on the ratio of organic mercury to total mercury content.

Well developed extraction and volatilization techniques are currently being investigated for the isolation of methylmercury from biological tissues. Since the possible radiolytic damage to organic mercury compounds during irradiation cannot be fully assessed, we are developing and applying a modified extraction technique to be used in a pre-irradiation and "normal" RNAA scheme. This will yield information about the changes of the chemical form of organic mercury during irradiation.

The separation of methylmercury is based on the volatilization of methylmercury cyanide formed in the reaction of methylmercury in a sample with hydrocyanic acid released by the interaction of a cyanoferrate with sulfuric acid at elevated temperatures. The methylmercury cyanide released is captured on cysteine paper in a microdiffusion cell. The paper is then placed in a flux of neutrons and the mercury quantitatively determined by neutron activation analysis.

The feasibility of this method is currently being tested. Szilard-Chalmers Reactions can change the chemical status of the mercury, and possible losses of mercury from the sample have to be assessed. Test analyses have proven that irradiation has to be performed in sealed quartz containers. Samples can then be counted either "instrumentally" in the irradiation container or after using the combustion methods. Both require comparable samples and standard configurations. The use of mercury deposits on filter paper (comparable to the proposed cysteine paper) is being evaluated.

In addition, the volatilization method for mercury determination after the reduction of the inorganic mercury by $SnCl_2$ will give the possibility of cross-checking of the mercury contents. The reduction method is claimed to be selective for the inorganic mercury (only 0.5% of the organomercury is released). This is, however, critically dependent on the chemical treatment of the samples. This has to be carefully investigated.

The procedure utilizes the volatility of mercury and its compounds, more importantly the inherent sensitivity of neutron activation analysis along with minimum use of reagents and solvents should provide increased accuracy over currently used methods.

SECTION 6

The Determination of Trace Elements in New Food Grain SRM's Using Neutron Activation Analysis

by

Thomas Earl Gills, Mario Gallorini, Harry L. Rook

INTRODUCTION

Increasing concern with possible toxic elements in the food chain plus growing recognition of the importance of a number of trace elements in nutrition make the accurate determination of trace elements essential (1). The National Bureau of Standards, in an interagency agreement with the Food and Drug Administration, is involved in developing and certifying selected elements in food grain as a part of the NBS Standard Reference Materials Program. The food grains chosen, rice flour and wheat flour, are currently being analyzed to determine trace element concentrations. Many of the elements considered to be toxic to human health are present at the ppb level, and their analyses require selective radiochemical separations. This paper presents results obtained using radiochemical and instrumental neutron activation analysis to determine thirteen trace elements whose effect on human systems ranges from toxic to essential. The following elements were determined in the food grain SRM's: Fe, Zn, Mn, Co, Na, K, Br, As, Sb, Se, Cu, Rb, and Hg.

EXPERIMENTAL

Samples and Standards:

Homogenized samples of Wheat and rice flours were vacuum dried at a pressure of 0.55 mm Hg for 48 hours in order to obtain a moisture content correction factor. For elemental standards, primary solutions were made by the dissolution of high purity salts or metals in NBS high purity acids (2).

Irradiations:

All samples and standards were sealed in Suprasil quartz vials and irradiated in the high flux pneumatic irradiation facility of the NBS Research Reactor. In this position the nominal neutron flux is 5×10^{13} $n \cdot cm^{-2} \cdot sec^{-1}$ with a Cu/Cd ratio of ~ 80. Within the irradiation container the radial flux variation is $\leq$ 2 percent (3).

Reagents for Radiochemical Separations:

Chromatographic reagents used for radiochemical separations consisted of inorganic ion exchangers developed by Girardi, et al. (4,5). Tin Dioxide (TDO), Cuprous Chloride (CUC) and Hydrated Manganese Dioxide (HMD) were obtained from Carlo Erba, Italy.

Counting:

All samples and standards were counted on a 66 cm^3 volume Ge(Li) detector coupled to a 4096 channel pulse height analyzer. For multiple analyses an automatic sample changer system was employed. After each count the accumulated data were transferred to a magnetic tape and were processed by the quantitative analysis program QLN (6).

INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS (INAA)

The following elements were determined by INAA: Br, Co, Fe, K, Mn, Rb, Zn, Na, and Se. The irradiation conditions and data used for the instrumental analysis are shown in Table 1. Different irradiation times were used to obtain the proper conditions for maximum sensitivity and accuracy. Approximately 300 mg of each food grain SRM, along with appropriate standards, were encapsulated in Suprasil quartz and irradiated in the high flux pneumatic tube facility of the NBS Reactor. After allowing the samples to decay for the specified time, samples and standards were post-weighed into fabricated polyethylene bags and counted. The resultant spectra were reduced to individual photopeaks associated with specific isotopes of interest and peak areas computed both by the total peak area method and by a peak fitting method. Elemental concentrations were determined by the direct comparator method.

Table 1. Irradiation Conditions and Data Used for Instrumental Analysis

Element Det'd	Isotope Measured	Half-Life ($T_{\frac{1}{2}}$)	Irradiation Time	Decay Time	Counting Time (sec)	Peaks Used for Evaluation (MeV)
Fe	^{59}Fe	44.6 d	8 hr	30 d	5×10^4	1.099, 1.292
Co	^{60}Co	5.27 y	8 hr	30 d	5×10^4	1.173, 1.332
Zn	^{65}Zn	244 d	8 hr	30 d	5×10^4	1.115
Zn	^{69M}Zn	13.9 h	8 hr	30 d	5×10^4	.439
Se	^{75}Se	120 d	8 hr	30 d	5×10^4	.136 .265
Rb	^{86}Rb	18.7 d	8 hr	30 d	5×10^4	1.077
Mn	^{56}Mn	2.58 h	3 min	2 hr	4×10^3	.847, 1.811
Na	^{24}Na	15.03 h	1 hr	30 hr	4×10^3	1.369, 2.754
Br	^{82}Br	35.34 hr	1 hr	30 hr	4×10^3	.776, .619
K	^{42}K	12.36 hr	1 hr	30 hr	4×10^3	1.525

RADIOCHEMICAL SEPARATIONS (RNAA)

The concentrations of arsenic, antimony, copper, selenium, and mercury were determined by RNAA. The irradiation conditions and data used for the radiochemical analysis are shown in Table 2. Inorganic ion exchangers were used in the determination of As, Sb, Se, and Cu while a combustion distillation procedure was used in the determination of Se and Hg. Complete descriptions and details of these procedures are given elsewhere (7-9).

Table 2. Irradiation Conditions and Data Used for Radiochemical Activation Analysis

Element Det'd	Isotope Measured	Half-life ($T_{1/2}$)	Irradiation Time (hr)	Decay Time (hr)	Counting Time (sec)	Peaks used for Evaluation (MeV)
As	^{76}As	26.4 hr	4	36	5×10^4	.559, .657
Cu	^{64}Cu	12.7 hr	4	36	5×10^4	.511, 1.345
Se	^{75}Se	120. d	4	36	5×10^4	.136, .265
Hg	^{197}Hg	64.1 hr	4	36	5×10^4	.077
Sb	^{122}Sb	2.7 d	4	36	5×10^4	.564
Sb	^{124}Sb	60.3 d	4	36	5×10^4	.603, 1.691

RESULTS AND DISCUSSION

The results of the instrumental analysis are shown in Table 3. The experimental design of the conditions for irradiation and counting was optimized to obtain the best statistical parameters. Under optimized conditions, the precision obtained was relatively good. The results of the analysis of NBS SRM Orchard Leaves 1571 also indicates relatively good accuracy.

The results obtained using radiochemical separation procedures are also summarized in Table 3. The values represent the averages obtained from the different radiochemical separation procedures.

The results shown constitute only one independent method of analysis (NAA) and are not considered NBS certified values. However, these results give a preliminary trace elemental profile of typical food grains (rice and wheat flour).

The procedures used in this work have been evaluated with respect to the sources of error that can affect the accuracy and precision of the results obtained.

Table 3. The Determination of Trace Metals in Rice and Wheat Flour in μg/gm

° INSTRUMENTAL ACTIVATION ANALYSIS

• RADIOCHEMICAL ACTIVATION ANALYSIS

Element	No. of Determinations	NBS Orchard Leaves SRM (1571) Certified*	NBS Orchard Leaves SRM (1571) Found	No. of Determinations	Wheat Flour Found	Rice Flour Found
°Bromine	6	(10)	9.7 ± 1.1	7	(9.9 ± 1.5)	(1.23 ± 0.08)
°Cobalt	6	(0.2)	0.14 ± 0.01	5	0.021 ± 0.004	0.018 ± 0.002
°Iron	6	0.030% ± 0.002	0.026% ± 0.002	5	17.2 ± 0.6	8.85 ± 0.94
°Potassium	6	1.47% ± 0.03	1.49% ± 0.03	7	1392 ± 37	1125 ± 16
°Manganese	12	91 ± 4	89 ± 5	12	8.6 ± 0.4	19.95 ± 0.69
°Rubidium	6	12 ± 1	11.7 ± 0.1	8	0.99 ± 0.16	7.27 ± 0.21
°Zinc	6	25 ± 3	24.8 ± 1.1	8	10.88 ± 0.56	19.97 ± 0.69
°Sodium	6	82 ± 3	84 ± 4	6	(10.4 ± 2.5)	(6.9 ± 0.4)
°Selenium	6	0.080 ± 0.01	0.086 ± 0.01	6	1.12 ± 0.01	0.42 ± 0.03
Arsenic	4	10 ± 2	10.0 ± 0.4	4	0.0054 ± 0.0005	0.40 ± 0.01
Antimony	4	2.90 ± 0.3	2.86 ± 0.08	4	0.038 ± 0.001	0.005 ± 0.001
Copper	4	12 ± 1	11.8 ± 0.30	4	2.0 ± 0.2	2.2 ± 0.13
Mercury	4	0.155 ± 0.015	0.154 ± 0.02	4	0.0010 ± 0.0003	0.0064 ± 0.001
Selenium	4	0.080 ± 0.01	0.084 ± 0.008	4	1.11 ± 0.05	0.45 ± 0.03

*Numbers in parentheses indicate information values only.

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Certain commercial equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental procedure. In no case does identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified is necessarily the best available for the purpose.

SECTION 7

Cadmium Analysis by Radiochemical Neutron Activation Analysis

by

Robert R. Greenberg, Mario Gallorini, Thomas E. Gills

Cadmium has been suspected of causing detrimental health effects in humans even at very low levels. Since Cd is commonly found at the trace or ultra-trace level, many analytical techniques do not possess the sensitivity to accurately determine the concentration of this element in many environmentally important materials. In addition, there are some instances in which the total amount of material available for analysis is very small, such as for certain types of atmospheric particulate samples, or hair samples. Analytical techniques capable of measuring very small amounts of Cd are therefore required.

Radiochemical neutron activation analysis (RNAA) was used at the National Bureau of Standards to analyze Cd in a variety of matrices. RNAA offers the advantages of high sensitivity, excellent selectivity, and no chemical blank. Furthermore, the ability to add carriers during the chemical dissolution and separation enables quantitative recovery of the element.

The concentrations of many elements can be determined instrumentally — that is, without separating them from the activated matrix. Other elements, however, such as Cd, usually have to be determined radiochemically, or isolated from other neutron-activated products. Many different types of radiochemical separations can be used for RNAA, such as: solvent extraction, distillation, precipitation, ion-exchange chromatography, and electrodeposition. The method we use to isolate Cd is solvent extraction. Zinc diethyldithiocarbamate [$Zn(DDC)_2$] in chloroform will quantitatively extract Cd from an aqueous solution over a pH range of from 1 to 12.

In addition to Cd, $Zn(DDC)_2$ also extracts Cu. In many matrices, Cu can interfere with the determination of Cd by producing a high background level of radiation. This problem can be eliminated by first extracting with bismuth

diethyldithiocarbamate [$Bi(DDC)_3$] in chloroform which removes Cu but not Cd. Cu can then also be analyzed if desired.

Preparation of Metal DDC Compounds

The $Zn(DDC)_2$ and $Bi(DDC)_3$ compounds are prepared by mixing aqueous solutions of NaDDC and either $Zn(NO_3)_2$ or $Bi(NO_3)_3$. The $M(DDC)_x$ compound formed is insoluble in water and precipitated. The precipitate is filtered, washed with water, and dissolved in chloroform. An equal volume of ethanol is added to the solution which is then set aside to allow the chloroform to evaporate at room temperature. After the chloroform evaporates, the $M(DDC)_x$ compound crystallizes in the remaining ethanol. The crystals are filtered and allowed to dry at room temperature.

Procedure

Prior to irradiation, the samples and primary standards were encapsulated in cleaned quartz vials. The standards consisted of a solution prepared from high purity metals dissolved in high purity HNO_3. The samples and standards were then irradiated together in the NBS reactor at a thermal neutron flux of 5×10^{13} $n \cdot cm^{-2} \cdot sec^{-1}$ for times ranging from one to four hours.

After irradiation, the radioactive samples were allowed to decay for three days after which they were transferred to dissolution vessels. Three types of vessels were used depending on the matrix: Erlenmeyer flasks, Teflon wet-ashing vessels and a Teflon-lined bomb. One hundred µg of Cu and Cd carriers were added to each vessel and the samples were dissolved. Various acid mixtures were used including HNO_3 alone, $HNO_3 - H_2SO_4$ and $HNO_3 - HClO_4$ (not in the bomb). If silica was present, some HF was also added.

After dissolution, 100 mg of Zn^{+2} hold-back carrier was added to minimize exchange between the radioactive Zn of the samples, and the organic Zn of the $Zn(DDC)_2$ solution used to extract the Cd. The pH of each solution was adjusted to 1.5 with NH_3 and the volume brought to about 60 mL with deionized water. Each solution was then transferred to a 125 mL separatory funnel and shaken for 30 minutes with 20 mL of 0.003 mol/L $Bi(DDC)_3$ in chloroform using a shaking machine. The organic fraction containing Cu was drained into a 120 mL polyethylene bottle and the aqueous phase was washed for 15 seconds with an additional 10 mL of the $Bi(DDC)_3/CHCl_3$ solution. The wash was then combined with the first $Bi(DDC)_3$ fraction and retained for counting.

Twenty mL of 0.005 mol/L $Zn(DDC)_2$ in chloroform was then added to the aqueous phase still in the separatory funnel, which was then shaken for five minutes. The organic fraction was drained into a second 120 mL polyethylene bottle, and the aqueous phase washed for 15 seconds with an additional 10 mL of the $Zn(DDC)_2/CHCl_3$ solution. The wash was combined with the first $Zn(DDC)_2$ fraction and was retained for counting.

Two different procedures were followed for the standards. One standard was prepared by pipetting a known amount of irradiated solution into a dissolution vessel with carriers and some unirradiated sample material. This material was dissolved in the same manner as were the samples used for analysis. A second standard was pipetted directly into 1 mol/L HNO_3 along with carriers. Both standards were then subjected to the same separation procedure used for the samples.

Counting

The samples and standards were counted on Ge(Li) detectors with active volumes of from 60 to 75 cm^3 coupled to 4096 channel pulse height analyzer systems. The $Bi(DDC)_3$ solutions containing Cu were counted immediately after separation. The 511 keV peak produced from the annihilation of positrons emitted by ^{64}Cu was used for analysis.

The $Zn(DDC)_2$ solutions containing Cd were allowed to decay for at least 24 hours to establish the equilibrium between ^{115}Cd and its daughter ^{115m}In. The 336 keV line from ^{115m}In, and the 527 keV line from ^{115}Cd were both used for analysis. Computer code QLN1 was used for data reduction supplemented by hand integrations of poorly defined peaks.

Results and Discussion

A large number of National Bureau of Standards Standard Reference Materials (SRMs) were analyzed by the above procedure, indicating the applicability of this method to a wide variety of matrices. The Cd and Cu results obtained are compared with the certified values in Tables 1 and 2. Very good agreement is observed demonstrating the accuracy of this technique.

Table 1. Cadmium in Various SRMs

	Concentration-μg/g	
	This Work	Certified
Orchard Leaves (SRM 1571)	0.116 ± 0.008	0.11 ± 0.01
Pine Needles (SRM 1575)	0.194 ± 0.009	< 0.5
Bovine Liver (SRM 1577)	0.295 ± 0.015	0.27 ± 0.04
Rice Flour (SRM 1568)	0.029 ± 0.005	0.029 ± 0.004
Wheat Flour (SRM 1567)	0.030 ± 0.005	0.032 ± 0.007
Sub-Bituminous Coal (SRM 1635)	0.030 ± 0.002	0.03 ± 0.01
River Sediment (SRM 1645)	10.2 ± 0.4	10.2 ± 1.5
Urban Particulate (SRM 1648)	71.2 ± 3.7	75 ± 7

This two-extraction radiochemical separation scheme is very versatile and can be used as a part of a larger multi-element analysis scheme. One such scheme used at NBS involves the use of an ion-exchange resin, HMD (Hydrated Manganese Dioxide) prior to the extractions. HMD will quantitatively retain As, Sb, Se, and Cr from a 1 mol/L HNO_3 solution. To perform this separation, we normally dissolve the sample in HNO_3 + $HClO_4$ with some HF if silica is present. The samples are heated to incipient dryness and then brought to a volume of 20 mL with 1 mol/L HNO_3. The solutions are passed through plastic chromatographic columns, each containing a bed of HMD 7 mm by 30 mm. The columns are washed twice with 1 mol/L HNO_3 and dismantled for counting. Under

Table 2. Copper in Various SRMs

	Concentration-μg/g	
	This Work	Certified
Orchard Leaves (SRM 1571)	11.6 ± 0.4	12 ± 1
Pine Needles (SRM 1575)	3.04 ± 0.16	3.0 ± 0.3
Bovine Liver (SRM 1577)	185 ± 7	193 ± 10
Rice Flour (SRM 1568)	2.12 ± 0.09	2.2 ± 0.3
Wheat Flour (SRM 1567)	2.21 ± 0.10	2.0 ± 0.3
Sub-Bituminous Coal (SRM 1635)	3.56 ± 0.18	3.6 ± 0.3

these conditions Cu and Cd are completely eluted. The eluted fraction is then subjected to the two-extraction procedure described above. Various SRMs were analyzed using this procedure and the results obtained are compared with the certified values in Tables 3-5. Very good agreement is observed.

Table 3. Sub-Bituminous Coal SRM 1635

	Concentration-μg/g	
	This Work	Certified
As	0.44 ± 0.05	0.42 ± 0.15
Sb	0.12 ± 0.01	(0.14)*
Se	0.82 ± 0.04	0.9 ± 0.3
Cr	2.48 ± 0.08	2.5 ± 0.3
Cd	0.030 ± 0.002	0.03 ± 0.01
Cu	3.56 ± 0.18	3.6 ± 0.3

*Values in parentheses are NBS information only values.

Table 4. Bovine Liver SRM 1577

	Concentration-μg/g	
	This Work	Certified
As	0.054 ± 0.004	(0.055)*
Sb	0.010 ± 0.002	---
Se	1.06 ± 0.06	1.1 ± 0.1
Cr	0.085 ± 0.009	0.088 ± 0.012
Cd	0.295 ± 0.015	0.27 ± 0.04
Cu	185 ± 7	193 ± 10

*Values in parentheses are NBS information only values.

Table 5. Orchard Leaves SRM 1571

	Concentration-μg/g	
	This Work	Certified
As	9.7 ± 0.4	10 ± 2
Sb	2.8 ± 0.1	2.9 ± 0.3
Se	0.09 ± 0.01	0.08 ± 0.01
Cr	2.67 ± 0.15	2.6 ± 0.3
Cd	0.116 ± 0.008	0.11 ± 0.01
Cu	11.6 ± 0.4	12 ± 1

Conclusions

The two-extraction radiochemical separation scheme for Cu and Cd described in this paper is both highly selective and extremely sensitive. The simplicity of the method allows a quantitative recovery of both elements, thus avoiding a calculation of chemical yield. Furthermore, a relatively short time is required to carry out the complete procedure. One person can separate about 15 samples a day.

Appendix I

Simultaneous Determination of Arsenic, Antimony, Cadmium, Chromium, Copper, and Selenium in Environmental Material by Radiochemical Neutron Activation Analysis

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A multielement radioanalytical procedure for the simultaneous determination of As, Cr, Se, Sb, Cd, and Cu has been developed. An inorganic ion exchanger coupled to a solvent extraction system was used for the selective separation of these elements from neutron activated matrices. The method has been used for certification of environmentally related NBS SRMs.

In studies of environmental pollution, elements such as arsenic, antimony, chromium, cadmium, copper, and selenium are among the most interesting because of their toxic nature. These elements are usually found at very low concentration in most samples of interest. The accurate analysis of these elements can be accomplished only by techniques that are very sensitive and selective. Neutron activation analysis usually meets these requirements; however, in many cases the elements to be determined must be separated selectively from neutron activated matrices. Utilizing radiochemical neutron activation analysis (RNAA), one can obtain maximum sensitivity, accuracy, and selectivity (*1*).

Usually, the choice of the radiochemical procedure to be followed is dependent upon the elements to be determined, the nature of the materials to be analyzed, and the simplicity of the chemistry. Several radiochemical procedures such as solvent extraction, distillation, ion-exchange chromatography, precipitation and electrodeposition can be used to obtain satisfactory results (*2–6*). However, when used alone, most of these procedures are not suitable for simultaneous multielement analysis. Furthermore, when several chemical steps are necessary, quantitative separation becomes extremely difficult; often the determination of chemical yields, which can contribute to the analytical error, is required.

In this work, a multielement radiochemical separation procedure has been tested and optimized to determine six elements simultaneously in different NBS environmental Standard Reference Materials (SRM's). Hydrated manganese dioxide (HMD), an inorganic ion exchanger developed by Girardi et al. (*7, 8*), was used for the retention and subsequent determination of As, Cr, Sb, and Se, while a solvent extraction system using diethyldithiocarbamate compounds (*9, 10*) was used to determine Cd and Cu.

In an attempt to demonstrate applicability to matrices having different chemical compositions, the following NBS materials were analyzed: Orchard Leaves (SRM 1571), Bovine Liver (SRM 1577), and Subbituminous Coal (SRM 1635).

EXPERIMENTAL

Reagents and Apparatus. The HMD inorganic exchanger was obtained from Carlo Erba, Milan, Italy.

Bismuth and zinc diethyldithiocarbamates (DDC) were prepared by mixing aqueous solutions of Na (DDC) with their respective nitrate salts (*9*). The $M(DDC)_x$ compounds formed precipitated from water. The precipitates were filtered, washed with water, and dissolved in $CHCl_3$. An equal volume of ethanol was added to the $CHCl_3$ solution which was then set aside to allow the $CHCl_3$ to evaporate at room temperature. After evaporation, the $M(DDC)_x$ compound crystallized in the remaining ethanol. The crystals were filtered and allowed to dry at room temperature.

Ion-exchange columns were made of polyethylene with an internal diameter of 7 mm and packed with 3 cm of hydrated manganese dioxide.

A Teflon-lined digestion bomb and Teflon wet-ashing vessels were used for sample dissolutions.

Standards and Carriers. A multielement primary standard was prepared from solutions of high purity metals dissolved in NBS high purity HNO_3 and/or H_2SO_4 (*11*). A multielement carrier solution was prepared by mixing solutions of the salts or

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Published 1978 by the American Chemical Society

Table I. Determination of As, Sb, Se, Cr, Cd, and Cu in NBS SRM Orchard Leaves (1571) and SRM Bovine Liver (1577)[a]

	matrice			
	NBS Orchard Leaves 1571		NBS SRM Bovine Liver 1577	
element	certified values	this work	certified values	this work
As	10 ± 2	9.7 ± 0.4	(0.055)	0.054 ± 0.004
Sb	2.9 ± 0.3	2.8 ± 0.1	(——)	0.010 ± 0.002
Se	0.08 ± 0.01	0.09 ± 0.01	1.1 ± 0.1	1.06 ± 0.06
Cr	2.60 ± 0.3	2.67 ± 0.15	0.090 ± 0.015	0.085 ± 0.009
Cd	0.11 ± 0.01	0.116 ± 0.008	0.27 ± 0.04	0.30 ± 0.02
Cu	12 ± 1	11.6 ± 0.4	193 ± 10	185 ± 7

[a] Concentration in μg/g. The reported value for each element consisted of ten determinations. Values in parentheses are NBS information values.

metals of interest dissolved in distilled water, HNO_3, and/or H_2SO_4. The concentration of each element in the multielement carrier solution was ~1 mg/mL.

The use of HCl or chloride salts was avoided to prevent possible losses of volatile chloride compounds during sample dissolution.

Sample Preparation. Special attention was given to sample handling prior to irradiation in order to avoid any possible contamination. Teflon-coated spatulas and forceps were used to transfer the samples into cleaned silica vials. Sample sizes ranged from 200–300 mg. Each irradiation capsule contained six vials, one liquid multielement standard, and five samples of the materials to be analyzed.

Irradiation. The standards and samples were irradiated in the RT-3 pneumatic tube facility of the NBS Research Reactor. In this position, the nominal neutron flux is 5×10^{13} n·cm^{-2} s^{-1}. The radial flux variation within the irradiation container is ≤2% (*12*).

Sample Dissolution. After irradiation and two days cooling time, the quartz vials were washed with concd HNO_3 and distilled water, cooled in liquid nitrogen to reduce the internal pressure, and opened. The contents were weighed and transferred to chemical dissolution containers along with 500 μL of the multielement carrier. Two types of dissolutions were performed to test for possible losses of volatile compounds. These were: (1) Dissolution in glass Erlenmeyer flasks or open Teflon wet-ashing vessels using 10 mL of concd HNO_3, $HClO_4$, and HF in a 10:3:1 ratio. (2) Dissolution in Teflon-lined bomb using 10 mL of fuming HNO_3 and HF in 20:1 ratio.

These acid mixtures were tested and found satisfactory for the subsequent radiochemical procedures.

Hydrated Manganese Dioxide (HMD) Separation. The solutions obtained from the Erlenmeyer flasks or Teflon vessels were evaporated to a final volume of approximately 1 mL, cooled, and brought to a 20-mL final volume with 1 M HNO_3. The solutions obtained from the bomb were evaporated in Teflon vessels to a volume of about 2 mL. One mL of concd $HClO_4$ was then added and the samples were heated to fumes of $HClO_4$, cooled, and brought to a final volume of 20 mL with 1 M HNO_3.

The 1 M HNO_3 solutions were then passed through HMD columns (7-mm diameter and 3-cm height) which had been preconditioned with 1 M HNO_3. The flow rate was adjusted to 0.5 mL/min by varying the packing density of the HMD. Selenium, chromium, arsenic, and antimony were retained on the HMD bed. Sodium-24, a major interference in both the biological and environmental matrices, passed through in the eluate. The columns were washed with 20 mL of 1 M HNO_3, dismantled, and the HMD was transferred to polyethylene counting vials while the eluted fraction was saved for the subsequent Cu and Cd separations.

Metal Diethyldithiocarbamate Extraction. The pH of the eluted fractions was adjusted to 1.5 with NH_4OH, and 100 mg of Zn holdback carrier was added. The solutions were then placed in separatory funnels and 20 mL of 0.003 M bismuth diethyldithiocarbamate [$Bi(DDC)_3$] in chloroform was added in order to extract the copper. The solutions were then shaken for 30 min, utilizing a shaking machine. The organic fractions containing the copper were placed in polyethylene bottles for counting. The cadmium remained in the aqueous fractions and was extracted with 20 mL of 0.005 M zinc diethyldithiocarbamate [$Zn(DDC)_2$] in chloroform. Five minutes of shaking time was found sufficient for a complete extraction of the cadmium complex into the organic phase. The resultant extract was also placed in polyethylene vials for counting.

Table II. Determination of As, Sb, Se, Cr, Cd, and Cu in NBS SRM Subbituminous Coal (1635)[a]

	Matrice	
	NBS SRM Subbituminous Coal (1635)	
Element	certified values	this work
As	0.42 ± 0.15	0.44 ± 0.05
Sb	(0.14)	0.12 ± 0.01
Se	0.9 ± 0.3	0.82 ± 0.04
Cr	2.5 ± 0.3	2.48 ± 0.08
Cd	0.03 ± 0.01	0.029 ± 0.003
Cu	3.6 ± 0.3	3.56 ± 0.18

[a] Concentration in μg/g. The reported value for each element consisted of ten determinations. Values in parentheses are NBS information values.

The Zn holdback carrier was added to minimize exchange between activated Zn from the sample and the organic Zn used for the Cd extraction. Radioactive Zn can interfere with the detemination of Cd by producing a high background level of radiation.

Standards. Standards for analysis were prepared in two ways. The first was prepared using the same procedure as used for the sample dissolution, HMD separation, and M(DDC)$_x$ extraction. The second standard was prepared by pipetting a known amount of irradiated standard solution directly onto HMD in a polyethylene counting vial for the determination of As, Sb, Se, and Cr. Copper and Cd standards were pipetted into 1 M HNO_3 acid and extracted in the same manner as the samples.

Counting. The samples and standards were counted on Ge(Li) detectors with active volumes of 63 to 75 cm^3 coupled to 4096-channel pulse height analyzers. The HMD fractions were counted twice; first, 4 days after irradiation for measuring short-lived radioisotopes of arsenic and antimony, and then 3 weeks after irradiation for measuring the long-lived radioisotopes of selenium and chromium. Because of the very low concentration of chromium in Bovine Liver, and the high background coming from ^{32}P β^- bremsstrahlung, counting times of from 5 to 10 h were necessary. The organic fractions containing the Cu were counted immediately after separation. The organic fractions containing the Cd were allowed to stand for 24 h to establish the equilibrium between ^{115}Cd-^{115m}In. γ rays from the decay of both these isotopes were used for Cd analysis.

Data reduction was accomplished by a quantitative analysis computer code (*13*) and hand integration of poorly defined peaks.

RESULTS AND DISCUSSION

The results are shown in Tables I and II. The results indicate very good agreement between certified and found values, demonstrating that the procedure is accurate.

During the dissolution of the samples in the Teflon bombs and in open Teflon containers, no appreciable losses of As, Cr, Se, Cu, and Cd were found. Large losses of antimony (up to 80%) were observed in some cases during the dissolution

in the open Erlenmeyer flasks due to the adsorption of Sb onto the glass surface of the flasks. The quantitative recovery of this element was impossible even with repetitive washing with 1 M HNO_3. No losses of antimony were observed, however, when the samples were dissolved in open Teflon vessels or in the Teflon-lined bomb.

A previous study by L. T. McClendon (*14*) cited apparent losses of organometallic chromium compounds during initial acid digestion. Because these volatile compounds were not characterized, all Bovine Liver samples analyzed in this work were digested in a Teflon-lined bomb. However, when the digested samples from the Teflon-lined bomb were boiled with $HClO_4$-HNO_3, no appreciable losses were observed. These observations give support to the theory that the first digestion in the Teflon bomb converts the organochromium compounds to inorganic chromium, which is not volatile in nonchloride systems. Furthermore, standards for analysis that were digested in $HClO_4$-HNO_3, and those pipetted directly were always in total agreement.

The retention of arsenic, selenium, chromium, and antimony on HMD in 1 M HNO_3 is highly selective and quantitative, while the elution of Cd and Cu is complete. The decontamination of the elements of interest from sodium-24 and other radioactive matrix interferences can be estimated to be a factor of 10^6.

CONCLUSION

The simplicity of the method permits a rapid isolation of the radioisotopes of interest without many chemical steps. The quantitative recovery avoids the calculation of the chemical yield. The sensitivity and accuracy obtained in this work demonstrate the validity and the precision of this radiochemical procedure.

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Received for review April 18, 1978. Accepted June 13, 1978.

Appendix II

The Quantitative Determination of Volatile Trace Elements in NBS Biological Standard Reference Material 1569, Brewers Yeast

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ABSTRACT

In the past few years, a large body of analytical data has been reported on trace levels of chromium in biological samples. From data on materials such as NBS Standard Reference Material 1577, Bovine Liver, and IAEA standard materials, it is now apparent that much of the reported Cr data are in error.

It has been suggested that some of the analytical problems may be due to the presence of a volatile organic complex of Cr in many biological matrices.

In an effort to resolve the question of Cr volatility, a series of experiments have been conducted on a new NBS Standard Reference Material--Brewers Yeast SRM 1569, which has been certified for Cr content. The experimental design allowed for the quantitative collection of volatile species in a thermally-heated vacuum distillation system over a temperature range of 150-325°C. A small fraction of the total Cu (<1%), about 25% of the total Hg and about 50% of the total Se were trapped and determined quantitatively. Arsenic, Ag and Au were also observed in the trapped fraction.

INTRODUCTION

The accurate determination of Cr in biological samples has historically been difficult. Analytical methods such as atomic absorption spectroscopy and neutron activation have the required sensitivity to determine ng levels of Cr but reported analytical data on biological materials, such as NBS Standard Reference Material (SRM) 1577 Bovine Liver, have been widely divergent (5). More recent data have indicated that the current status of Cr analysis has not greatly improved (6). It has been suggested that part of the analytical error may be due to the presence of a volatile organic species which may be lost in certain sample-preparation procedures (7,8).

In general, analytical verification of volatile trace elemental species in biological systems has been limited. Elements such as Hg and Pb have been shown to form elemental or simple molecular species which vaporize upon mild thermal treatment. The awareness and confirmation of possible vaporization losses of other metals which are not normally considered volatile is becoming of increasing importance. Two recent studies have reported no Cr losses during ashing and acid digestion of

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biological samples to which radiotracers of Cr-51 were added (5,6). A more recent study has reported as much as 30% volatilization loss of Cr from neutron activated brewers yeast samples during dissolution with concentrated acids (4).

In an effort to resolve the question of Cr volatility, a vacuum distillation experiment has been designed and conducted on a new NBS Standard Reference Material, SRM 1569 Brewers Yeast, which is certified for total Cr content. The experimental design enabled volatile species to be collected from samples heated in the range of 150-325°C, and for the quantitative determination of those elemental species amenable to neutron activation. In addition to Cr volatility losses were found and quantified for mercury, Se and As. Other elements such as Br and I were observed to volatilize but were not quantified due to experimental conditions of the NA procedure.

MATERIALS AND METHODS

The basic experimental system is pictured in Figure 1. The system consists of a tube furnace (Lindberg, Type JJ035 Hevi-Duty, 300W, Watertown, WI*) used to heat a 19 mm i.d. quartz combustion tube containing the sample and a thermometer. One end of the quartz tube was closed with a standard taper ground glass removable stopper. The other end was fitted with a second standard taper joint, to which was sealed a 30-cm piece of 8 mm i.d. quartz tube. This extension was passed straight through a liquid nitrogen (LN_2) trap and was used to collect the distillate. The trap was constructed of styrofoam with holes bored through the sides to seal to the collection tube. The collection tube was connected to a vacuum pump with rubber vacuum tubing. A second liquid N trap was placed between the pump and the collection tube to prevent back streaming of pump oil vapors and other contaminants.

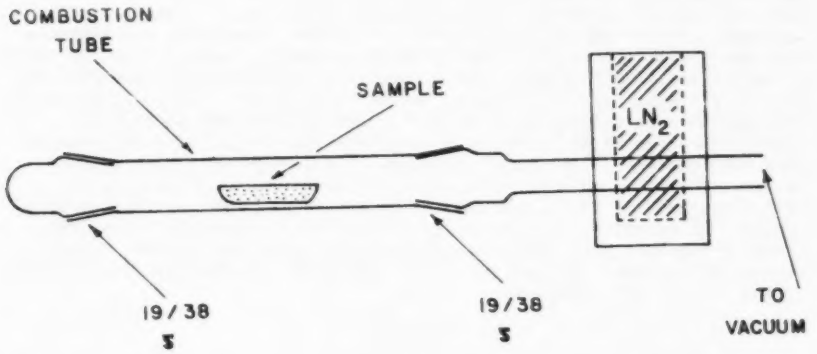

FIGURE 1— VACUUM AND COLLECTION SYSTEM.

In conducting each experiment, the quartz collection tube was first rinsed several times with ultrapure HCl and HNO_3 to remove trace element

*In order to adequately describe materials and experimental procedures, it was occasionally necessary to identify commercial products by manufacturer's name or label. In no instance does such identification imply endorsement by the National Bureau of Standards nor does it imply that the particular products or equipment is necessarily the best available for that purpose.

contamination from the inner surface (3). The combustion tube was also cleaned and purged by heating to 800°C with a low flow of oxygen through it. The collection tube was then sealed to the sample tube and the system again purged. Following the precleaning procedure, approximately one g of NBS SRM 1569 Brewers Yeast was weighed into an aluminum foil sample boat. The sample was placed into the combustion tube along with a thermometer whose bulb rested against the sample boat. The collection tube was then connected to the vacuum system, liquid N was added to the collection trap, and the system was evacuated.

The furnace was preheated to the desired temp and placed around the sample tube. The entire system was then heated to the desired equilibration temp and maintained at that temp (± 5°C) for 15 min. During this 15-min period the entire system, up to the LN_2 condenser, was heated gently with the oxygen-gas torch to prevent condensation of the vapors outside of the collection trap. Care was taken to prevent decomposition of the vapors due to excessive heat in the tranfer line. The volatilized material from the sample was collected in the liquid N trap portion of the collection tube. For several of the higher temp runs, the trap had to be periodically moved several mm closer to the sample tube to prevent a solid plug of condensed material from forming inside the collection tube. All of the condensed material, however, remained at liquid N temp throughout the run.

At the end of the 15-min collection period, the furnace was removed and the combustion tube allowed to cool. The collection tube, while still under vacuum, was sealed off between the combustion tube and the liquid N collection trap. The other end of the collection tube was then sealed with the sample still frozen and under vacuum. The sealed samples were then set aside for irradiation and analysis.

The analytical portion of the experiment was conducted by non-destructive neutron activation. For analysis, 4 of the sealed quartz tubes containing the condensed volatile material were irradiated together with one quartz tube containing 300 µl of a mixed trace element water standard. The 4 samples were placed in a single irradiation container so that one sample from each of the temperature levels, ranging from 150-300° C, were irradiated together. The samples and standard were irradiated for 4 hr at a thermal neutron flux of ~ 6×10^{13} n-cm^{-2}sec^{-1}. After irradiation, the samples were allowed to decay for 2 days to eliminate short half-lived radioactivity and counted on a large volume Ge(Li) detector coupled to a 4096 channel pulse height analyzer. The samples were then allowed to decay for 2 wk and were recounted to determine those elements with half lives greater than a few days. The resultant gamma-ray spectra were recorded on magnetic tape and processed off-line by computer. The individual photopeaks were quantified by the total peak area method. Quantitative results were obtained by direct comparison of the specific activity of the samples and standard.

RESULTS

Detectable amounts of 6 trace elements were observed in trapped fractions distilled from the brewers yeast. The amount of each element trapped was dependent upon the maximum temp to which the sample was heated. The quantities of those trace elements observed were normalized to the weight of yeast used in individual experiments and are given in Table I. The amount of each element in the trapped fraction increased with increasing temp in all cases except Ag. However, the quantity of the element volatilized and the dependence on temp varied among the individual elements, as shown graphically in Figures 2-5.

The data in Table II reflect elemental concentrations in the yeast residue relative to those in the original unheated yeast sample. These data are corrected for total wt loss of material from the sample due to

vacuum distillation. The elemental data are given relative to initial yeast concentration because the absolute trace element content of the SRM Yeast has been determined only for Cr. Approximately 50% of the total Se, about 25% of the total Hg and less than 1% of the total Cr content were lost at the 300°C temp. The total weight loss of material was between 30 and 40% at that temp. For Cr these data are consistent with the observed volatility data. Although a volatile Cr component was observed, the quantity, ~.003-.005 µg/g, was within measurement error of the total certified Cr content in the yeast. Scandium, Fe and Co were not detected in the trapped fraction and no differences were observed in any residue samples compared to initial samples.

TABLE I. TRACE ELEMENTAL LOSS FROM BREWERS YEAST BY VACUUM DISTILLATION

	µg trapped/g sample Temp °C					
Element	150	190	205	250	295	305
Hg	0.0014	0.006	---	0.017	0.10	0.055
Se	0.0013	0.0027	0.0018	0.036	0.16	0.23
Cr	0.0028	0.0021	0.0021	0.0050	0.0038	0.0052
As	0.064	0.060	0.050	0.10	0.22	0.32
Ag	0.0045	0.0005	0.0028	0.0027	0.0010	0.0042
Au	0.00010	---	0.00006	0.00040	---	---

TABLE II. RELATIVE ELEMENTAL COMPOSITION OF YEAST RESIDUE

	Concentrations Relative to Unheated Yeast Temp °C					
Element	0	0	200	265	290	305
Sc	0.996	1.01	0.992	1.00	1.00	1.02
Fe	1.00	0.983	1.00	1.00	1.01	0.983
Co	1.00	1.00	0.905	1.01	0.910	0.933
Cr	1.01	0.990	1.06	0.920	1.02	1.06
Hg	0.990	1.01	xxx	1.01	1.06	0.74
Se	1.04	0.960	1.12	0.64	0.44	0.57
% wt loss	0	0	7%	22%	39%	32%

DISCUSSION

The appearance of detectable amounts of several trace elements in the vacuum distillate of a sample of brewers yeast suggests the presence of volatile elemental species in biological materials. For some metals such as Hg, these species may volatilize at temps lower than 150°C, while for the other elements observed in this work the volatilization began between 200 and 250°C. This is also the temp at which visible charring of the brewers yeast occurred (Table II). Thus, the volatility and loss of these elements appears in general to be more of a thermal degradation of the matrix with concomitant release or decomposition of the naturally occurring metallic species, resulting in a volatile product. The

analytical significance of these losses, especially in methods which rely upon thermal decomposition of the organic matrix prior to solubilization and analysis, is obvious. For example, the data from this study indicate that up to 50% of the total Se content can be volatilized upon moderate heating.

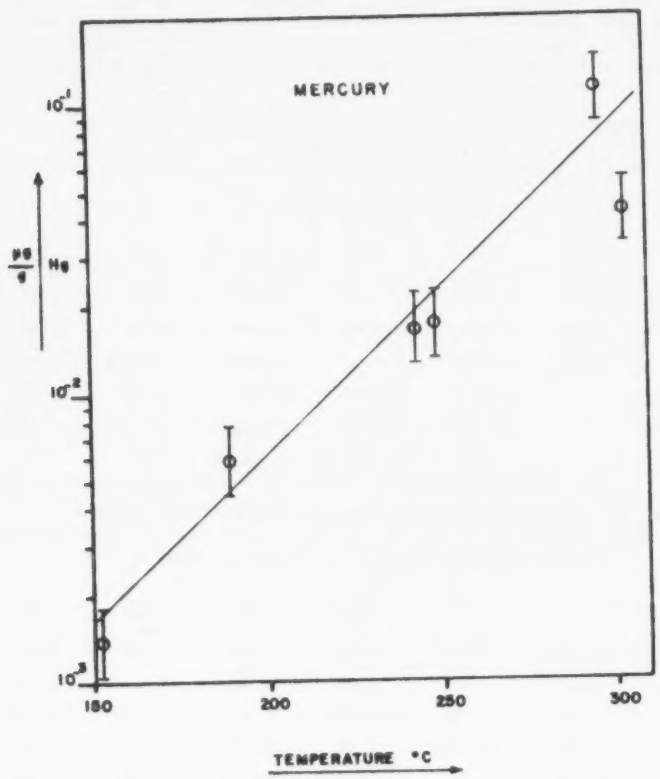

FIGURE 2– COLLECTED VOLATILE MATERIAL VS. TEMPERATURE-MERCURY.

Figures 2-5 show some very interesting trends. If one assumes that the ideal pattern for volatilized material would follow a curve as depicted in Figure 6, there is a temp, A, below which no loss occurs. At temp A, losses begin due to natural volatility or thermal degradation. At any temp above A, the amount of volatilized trace element will be determined by the rate of formation, vapor pressure of the volatilized species and the length of time at that temp. In this experiment the length of time at the maximum temp was held constant; therefore, the amount of material volatilized was dependent upon the rate of formation of the volatile species, up to the limiting total amount of that species or its precursor. At point B in Figure 6, the limiting condition has been reached and no more material is volatilized.

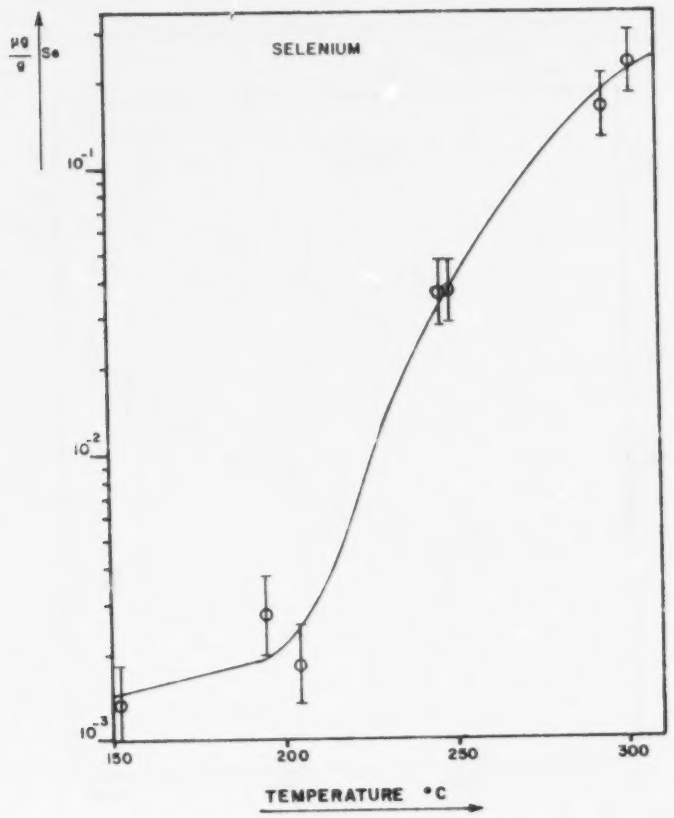

FIGURE 3– COLLECTED VOLATILE MATERIAL VS. TEMPERATURE-SELENIUM.

If we examine the experimental data for the different elements in terms of this idealized curve, a consistent trend is apparent. For Hg (Fig. 2) we see a logarithmic increase throughout the temp range studied, indicating that we are between transition points A and B for this element. The initial volatilization temp for Hg is below 150°C. At 300°C, a significant portion of the total volatile Hg was not vaporized in the 15-min distillation period; thus point B had not been reached. This interpretation is consistent with the Hg loss data in Table II.

The data for Se (Fig. 3) show the entire curve as idealized. The initial volatilization point is around 175°C and we see that the amount of trapped Se appears to be reaching a maximum at 300°C. Thus, a significant portion of the total volatile species has been vaporized in 15 min at 300°C. Again, the loss data in Table II are consistent in that approximately 50% of the total Se in the sample was lost. This implies that there are at least 2 different fractions or species of Se in the brewers yeast and that they are approximately equal in amount. With the knowledge that different chemical species or forms may have widely differing effects

or functions in biological systems, this confirmed multiplicity of Se species is of great significance.

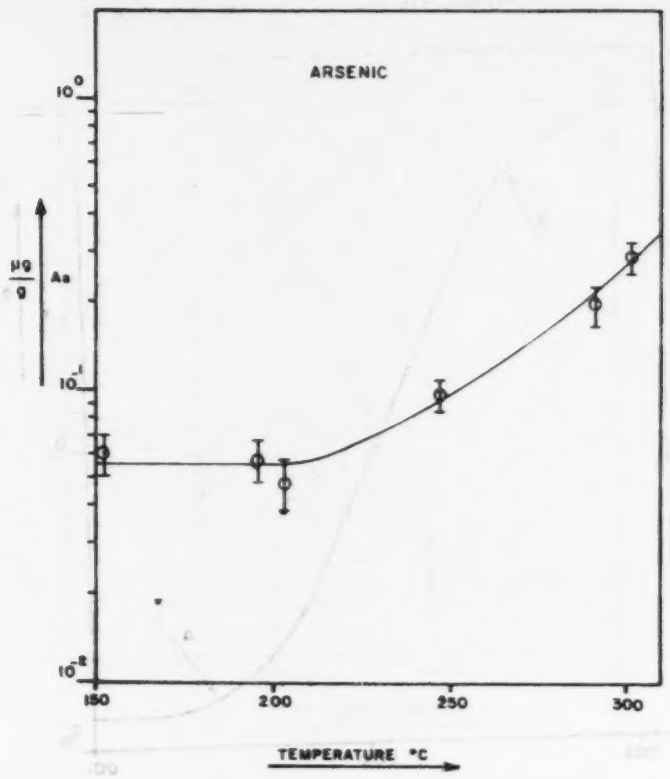

FIGURE 4— COLLECTED VOLATILE MATERIALS VS. TEMPERATURE-ARSENIC.

For As (Fig. 4) we see the beginning of the idealized curve with transition point A at about 200°C. At the maximum temp of 300°C we have not yet volatilized a significant portion of the total As species as the curve is still increasing logarithmically.

The amounts of trapped Cr (Fig. 5) are significantly higher for the samples collected above 200°C than for those collected below that temp although the absolute amounts are very low. These data show that about 5-10 ng/g of Cr is volatilized by thermal degradation between 150°C and 300°C. Although this is a very small fraction of the total content, it signifies a fraction of Cr in this sample which is chemically different from the bulk. It has been shown that determination of Cr in biological materials by thermal heating and atomization in graphite furnaces is very susceptible to chemical form and matrix in which it occurs (7,8). This existence of a fraction of Cr that can be directly distilled supports those earlier findings. In the studies which reported no losses of Cr during ashing and acid digestion with added radiotracer (1,2) there was no

assurance that the Cr-51 tracer was present in, or had exchanged with, the organic forms of Cr in the samples. Also, the experiments were designed to determine the amount of Cr retained in the sample after treatment, but were not specifically designed to determine small Cr losses via volatilization. In the recent study reporting loss of up to 39% of the Cr during dissolution with concentrated $HClO_4$ and HNO_3, mixtures were collected and the losses due to volatility were quantitatively verified (4). Due to the prior neutron irradiation and the severe conditions of acid dissolution, it is highly probable that the Cr species originally present in the sample was changed during the procedure. In our studies, the sample was subjected to only a thermal stress and no chemical treatment was carried out. These studies do reemphasize the importance of initial chemical speciation and matrix upon the potential for formation of volatile species during thermal or chemical reactions.

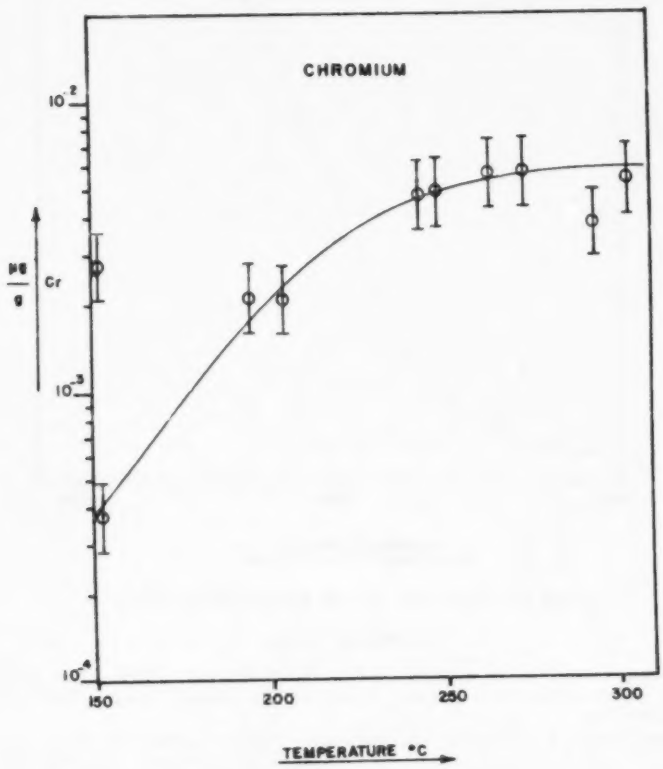

FIGURE 5– COLLECTED VOLATILE MATERIAL VS. TEMPERATURE-CHROMIUM.

In summary, we have shown that chemical species of several trace elements exist in brewers yeast which can be volatilized by thermal heating under vacuum distillation, and that the temperature dependence of the quantitative distillation varies among the different trace elements.

We have also shown that in a given biological material, some trace elements exist in more than one chemical state. The potential exists for use of this type of differential pyrolysis or fractional distillation in studies of chemical speciation of these trace elements.

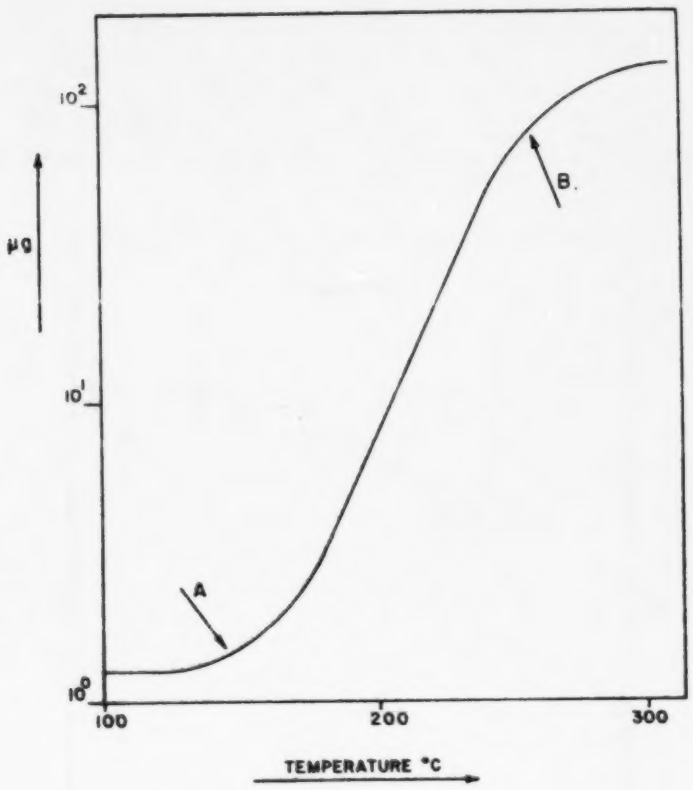

FIGURE 6— IDEALIZED VACUUM DISTILLATION CURVE.

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DISCUSSION

Inquirer: W. R. Faulkner, Vanderbilt University, Nashville, TN

Q. Is it likely that vol Cr might be appreciable at temps greater than 305°C and less than charring temps?

A. Not likely because Cr conc is beginning to plateau at temps less than 305°C. There was difficulty because of clogging of trap at temps greater than 305°C.

Inquirer: Richard A. Peabody, V.A. Hospital, Albany, NY

Q. Has chromyl chloride been observed directly in your analytical system for volatile components, presumably formed by interaction of HCl with Cr in digests?

A. No, not in our system. The procedure used in this study was vacuum distillation of volatile materials directly from the brewers yeast. No acid digestion, using HCl or any other acids, was used. Also, the neutron activation technique used to quantify the amounts of trace elements volatilized would not detect chemical compounds, such as chromyl chloride; it is a total elemental technique.

Appendix III

Chemical Preparation of Biological Materials for Accurate Chromium Determination by Isotope Dilution Mass Spectrometry

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National Bureau of Standards
Washington, D. C.

ABSTRACT

The current interest in trace elements in biological materials has created a need for accurate methods of analysis. The source of discrepancies and variations in chromium concentration determinations is often traceable to inadequate methods of sample preparation. Any method of Cr analysis that requires acid digestion of a biological matrix must take into consideration the existence or formation of a volatile Cr component. In addition, because Cr is often present at concentrations less than 1 µg/g, the analytical blank becomes a potential source of error.

Chemical procedures have been developed for the digestion of the biological matrix and the separation of Cr without either large analytical blanks or significant losses by volatilization. These procedures have been used for the analysis of NBS Standard Reference Material (SRM) 1569 Brewers Yeast; SRM 1577 Bovine Liver; SRM 1570 Spinach and other biological materials including human hair and nails. At this time, samples containing 1 µg of Cr can be determined with an estimated accuracy of 2%.

INTRODUCTION

The accurate determination of chromium in biological materials presents a considerable challenge to the analytical chemist. Because the concentration of Cr is often less than 1 µg/g, it is imperative that the analytical method be sensitive and non-contaminating. Isotope dilution mass spectrometry (IDMS) is capable of precise and accurate concentration determinations on submicrogram amounts of an element, and as a result, a major limitation of this method is the magnitude and variability of the analytical blank. Through the use of clean room facilities, pure reagents and apparatus fabricated from quartz and Teflon, the variability of the Cr analytical blank can be maintained at a level of less than 10 ng. Control of the analytical blank at this level permits the determination of 1 µg of Cr with an estimated accuracy of $\pm$2%.

For biological samples, accuracies of this magnitude are not only dependent on controlling the analytical blank, but on preventing loss of the volatile Cr compounds during the wet oxidation of the matrix. The presence of volatile Cr compounds in various biological materials has been discussed (3,4) and it is suspected that many of these compounds may volatilize at low temperatures. In addition, the formation of volatile Cr compounds during the wet oxidation process must be avoided. For this reason, perchloric acid should not be used since the formation of chromyl chloride is likely to produce losses of Cr. The loss of other volatile components can be controlled by performing the wet oxidation procedure in a quartz reflux system.

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Once the digestion of the matrix is complete and the spike and natural Cr have equilibrated, it is no longer necessary to maintain complete recovery. However, recoveries greater than 80% are essential for the accurate determination of ng amounts of Cr, since a few ng of blank are sufficient to significantly bias the concentration measurement. Thus, understanding and controlling the effects of the analytical blank and the loss of volatile Cr compounds are the major factors in the accurate determination of Cr in biological materials by IDMS.

MATERIAL AND METHODS

The dissolution, separation and purification of all samples were performed in a Class 100 clean air environment to minimize particulate contamination. With the exception of the ceric sulfate solution, all reagents used for these analyses were purified by sub-boiling distillation (2). The ceric sulfate was prepared from high purity cerium (IV) oxide by several evaporations with sulfuric acid.

The ^{50}Cr spike, a 96% enriched isotope, was calibrated by comparison with 2 different solutions of high purity natural Cr. Weighed aliquots of the spike and natural solutions were mixed and equilibrated and the concentration of the spike was determined by IDMS.

The quartz reflux system used for the wet oxidation of the organic matrix consisted of a 125 ml Erlenmeyer flask fitted with a quartz condenser. The refluxing action was aided by filling the condenser with quartz chips and by running chilled water through the condenser jacket.

The chemical separation procedure is similar for all biological materials discussed in this paper. The sample is placed in a quartz Erlenmeyer flask and ^{50}Cr spike is added to alter the natural $^{50}Cr/^{52}Cr$ ratio. Sample wts ranging from 1 g for spinach and brewers yeast up to 5 g for blood serum were used. The flask is then fitted with a condenser and chilled water is run through the jacket for approximately 1/2 hr. Ten g of concentrated (70%) nitric acid are added through the top of the condenser and the temp is increased gradually to avoid excessive foaming and frothing of the sample. After any initial reactions have ceased, 9 g of concentrated (98%) sulfuric acid are added. This mixture is digested for approximately 5 hr while gradually increasing the temp to ~250°C. The condenser jackets are then drained and the refluxing action is continued overnight. In cases where silica is present in the matrix (e.g. brewers yeast and spinach), the contents of the flask are transferred to Teflon beakers and treated with 1 g of concentrated (36%) hydrofluoric acid.

The Cr is then oxidized with ceric sulfate, separated by extraction with methyl isobutyl ketone and back extracted into water. The resulting solution is evaporated to dryness and the purified Cr is analyzed by surface ionization mass spectrometry.

All isotopic ratio measurements were made using a 15-cm radius of curvature, solid sample, single focusing mass spectrometer. The details of the tungsten filament surface ionization procedure for Cr will be published (1). All isotopic ratio data were corrected to the absolute isotopic composition of a Cr isotopic reference standard (SRM 979) which was analyzed under the same conditions as the samples.

RESULTS AND DISCUSSION

The results of Cr determinations on various biological materials are shown in Tables I-III. The effect of the blank varies with the concentration level, and as the amount of Cr determined approaches the submicrogram range, the accuracy of the method becomes primarily dependent upon the magnitude and variability of the blank. The uncertainty for the determination of Cr in Brewers Yeast SRM 1569 (Table I) is the estimated accuracy and includes allowances for the precision of the ratio measure-

ment, the calibration of the ^{50}Cr spike, the variability of the Cr analytical blank and sample inhomogeneity.

TABLE I. CHROMIUM CONCENTRATION IN SRM 1569 BREWER'S YEAST

Bottle No.	μg Cr/g
5	2.07
127	2.10
128A	2.09
15A	2.08
340	2.23
253A (1)	2.13
253A (2)	2.12
Average	2.12 ± 0.13[a]

[a]The uncertainty (ts) is the estimated accuracy and includes allowances for the precision of the ratio measurement, the calibration of the ^{50}Cr spike, the variability of the analytical blank and sample inhomogeneity.

TABLE II. CHROMIUM CONCENTRATION IN SRM 1577 BOVINE LIVER

Bottle No.	No. of Determinations	ng Cr/g
1	4	95.6
2	3	85.3
3	2	89.3
4	4	97.8
	Average	92.0[a]

[a]Analytical blanks averaged 13 ± 7 ng

TABLE III. CHROMIUM CONCENTRATION IN OTHER BIOLOGICAL MATERIALS

Matrix	μg Cr/g
SRM 1570, Spinach	4.67
Hair	2.65
Toenails	2.93
Blood serum	0.008

Since both SRM 1569 Brewers Yeast and SRM 1577 Bovine Liver were suspected of containing volatile Cr compounds, an attempt was made to check for possible losses. This process involved taking additional samples of each SRM, which were spiked after the wet oxidation of the matrix had been completed. Any losses of Cr up to this point in the analysis should produce a measured ratio depleted in natural Cr and thus yield lower concentration data. In both cases discussed above, the Cr concentrations determined by spiking before and after dissolution were within the estimated uncertainty of the analysis and as a result it would appear that if a volatile constituent is being lost, the amount is probably very small.

The bovine liver data in Table II exemplify the analytical problems encountered when analyzing ng levels of Cr. These measurements were part of a preliminary experiment to evaluate the IDMS technique for Cr in the bovine liver matrix and to test for sample inhomogeneity. Two-g samples of the liver were analyzed so that the total amount of Cr was approximately 200 ng. However, because the variability of the analytical blank was ± 7 ng, the accuracy was estimated to be $\pm 4\%$ for each determination. At this concentration level, the effect of even relatively small changes in the blank made it difficult to ascertain the cause of sample variations.

Table III contains a summary of concentration determinations on other biological materials. The blood serum represents the lowest Cr concentration determined to date. Although 5-g aliquots of serum were taken for analysis the magnitude of the analytical blank was 25% of the total Cr in the sample.

CONCLUSION

By employing techniques that minimize the magnitude and variability of the analytical blank as well as losses of volatile Cr compounds, the concentration of Cr in biological materials can be accurately determined. Although the magnitude and variability of the analytical blank becomes increasingly significant as the amount of Cr approaches the low ng range, uncertainties of 2% or less are possible for the determination of at least one µg of Cr.

ACKNOWLEDGMENTS

We wish to express our appreciation to Joy J. Shoemaker and Karen A. Brletic for manuscript preparation, to William A. Bowman,III for instrument maintenance support and to our colleagues of the Analytical Spectrometry Section for their helpful discussions.

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Appendix IV

The Determination of Zinc, Cadmium and Lead in Biological and Environmental Materials by Isotope Dilution Mass Spectrometry

J. W. Gramlich, L. A. Machlan
T. J. Murphy and L. J. Moore

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ABSTRACT

Techniques have been developed for the accurate and precise determination of the concentration of zinc, cadmium and lead using thermal ionization mass spectrometry. These techniques have been applied to the analysis of a variety of biological materials such as blood, hair and nails, and to environmentally related materials such as water, air particulates and fossil fuels.

Uncertainty in the accuracy of the isotope dilution method is less than 0.5% for all 3 elements, and is nearly independent of element concentration from the % level down to the ng/g (ppb) range where the analytical blank becomes a significant contribution to the uncertainty.

The mass spectrometric methodology and chemical separation procedures are presented along with examples showing the effect of sample impurities on the quality of the analytical data.

INTRODUCTION

The toxicity of cadmium and lead, and the associated hazards resulting from increased pollution of the environment by these elements, has received considerable attention during the past decade. The serious pathological effects of Cd (3,8) and the hematological disturbances associated with lead poisoning (2) have been extensively reviewed in the literature. Zinc is an essential element for growth and maintenance of body functions; it is a constituent of several enzymes and a cofactor for certain enzymatic reactions. There is ample evidence to suggest that the metabolisms of Cd and Zn are closely related and that Cd has the ability to exchange with Zn and thus produce changes in enzymatic activity (3). The close biochemical and geochemical association of Zn and Cd make their collective study in biological and environmental systems highly desirable.

As the concentration of these trace elements approaches the ng/g (ppb) range, it becomes increasingly difficult to maintain accuracy and precision in routine analytical measurements. Well characterized trace element standard materials are essential to ensure quality control and to develop or verify analytical methods. Although not suited for routine analysis because of the time and expense involved, isotope dilution mass spectrometry provides an extremely valuable tool for trace element analysis of standard materials, for refereeing discrepancies between other analytical methods and for determining small sample inhomogeneities.

Samples to be analyzed are spiked with a known amount of a separated isotope of the element of interest. After dissolution of the sample and equilibration of sample and spike, quantitative recovery is no longer necessary because only the altered isotopic ratio is of interest. Herein

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lies a major advantage of the isotope dilution technique over other analytical methods. Because only a representative portion of the sample is needed for analysis, rigorous chemical purification can be employed to obtain a high purity, matrix-free sample that can be compared directly with pure standards of known absolute or relative isotopic abundances.

The high degree of sensitivity afforded by the mass spectrometric method allows the concentrations of Zn, Cd and Pb, as well as many other elements, to be determined with both high accuracy and precision at concentrations below one mg/g. The practical lower limit of concentration measurements is governed by the magnitude and variability of the analytical blank.

MATERIALS AND METHODS

Accurate mass spectrometric measurements require a high purity sample for analysis. Trace amounts of impurities may result in reduced signal intensity and alteration of the filament fractionation pattern (4). In addition, isobaric interferences from Ge, Ni, Pd, Sn and In may affect the determination of Cd and Zn. Although the exact chemical separation and purification procedures may vary somewhat, depending on the particular matrix, the following procedure is generally applicable. A known amount of sample is spiked with weighed aliquots of ^{67}Zn, ^{111}Cd, and ^{206}Pb solutions. The sample is then digested with a mixture of hydrochloric, nitric and perchloric acids. After dissolution and equilibration of the sample and spike, the 3 elements are separated from each other and the matrix by anion exchange chromatography. Further purification of Pb and Cd is accomplished by electrodeposition: Pb by anodic deposition as PbO_2 (1) and Cd by cathodic deposition from an ammoniacal solution. Further purification of Zn is frequently necessary to remove traces of Fe which interfere with the mass spectrometric analysis. This is accomplished by additional anion exchange chromatography.

Since the limiting factor for the accuracy of trace element analysis is often the magnitude and variability of the analytical blank, all chemical preparation is conducted in a clean room facility (7) using acids and water prepared in this laboratory by sub-boiling distillation (5). A class 100 clean air environment is also utilized to dry the samples on the mass spectrometer filaments.

All analyses reported here were performed on 30 cm radius, 90° magnetic sector mass spectrometers utilizing thin lens z-focusing sources and Faraday cage collectors equipped with 50% transmission grids shadowing a series of suppression grids (10). The suppression grids are designed to provide cubic suppression of secondary particles between the transmission grid and the collector-defining slit. The remainder of the measurement circuits consisted of a vibrating reed electrometer, voltage to frequency converter, scaler and programmable calculator. Timing, magnetic field switching and data acquisition were controlled by the programmable calculator.

Zinc, Cd and Pb were analyzed from separate loadings onto single filament rhenium sources employing the silica gel-phosphoric acid technique for ionization enhancement (1,9).

RESULTS AND DISCUSSION

The concentration of Pb at or below the μg/g level has been routinely determined in this laboratory for several years. Samples relevant to this discussion include porcine, bovine and human blood (0.03-1 μg/g), human hair and nails (28 and 1.6 μg/g respectively), botanical materials (1-10 μg/g), gasoline (12-700 μg/g) and water (0.02-1 μg/g). The determination of Zn and Cd concentrations by isotope dilution has been recently undertaken, based on results which indicated that sufficient signal intensities could be generated using the silica gel-phosphoric acid technique (6,9).

Cadmium produces signal intensities and stabilities comparable to those obtained with Pb; however, the ionization appears to be easily poisoned by trace impurities, especially Zn. Electrodeposition as a final purification step has been found necessary to obtain maximum signal intensity. The analytical blank for Cd is less than 1 ng, which is substantially better than the typical 1-5 ng blanks for Pb. Measurement precision at present appears to be in the range of 0.1%-0.2% (see Table III).

Zinc produces a signal intensity at least an order of magnitude lower than Cd or Pb. This fact, combined with relatively high and variable blanks, restricts the practical concentration limits to samples containing more than 100 ng of Zn. Fortunately, Zn is abundant in nature and samples below the µ/g level are not prevalent. Further research is expected to identify and reduce the sources of the blank.

The results obtained by isotope dilution mass spectrometry on 2 new candidate NBS Standard Reference Materials are reported in Tables I and II. These SRMs should be of particular interest to those involved in environmental monitoring.

TABLE I. ZINC, CADMIUM AND LEAD IN URBAN PARTICULATES (SRM 1648)

Bottle No.	Percent Zn	µg Cd/g	Percent Pb
40	0.4769	76.06	0.6597
148	0.4784	75.65	0.6590
162	0.4730	77.98	0.6595
757	0.4768	75.95	0.6598
808	0.4741	76.14	0.6579
1636	0.4761	76.05	0.6611
Average	0.4759	76.31	0.6595
Std. Dev.	0.0019	0.84	0.0010
Range	0.0054	2.33	0.0032

TABLE II. ZINC, CADMIUM AND LEAD IN COAL
(Cadidate NBS SRM 1632a)

Bottle No.	µg Zn/g	µg Cd/g	µg Pb/g
1	26.94	0.1688	12.21
2	26.88	0.1698	12.35
16	27.09	0.1692	12.10
93	27.82	0.1746	12.33
Average	27.18	0.1706	12.25
Std. Dev.	0.43	0.0027	0.12
Range	0.94	0.0058	0.25

TABLE III. REPETITIVE MASS SPECTROMETRIC ANALYSES OF ZINC, CADMIUM AND LEAD IN COAL - BOTTLE NO. 1
(Candidate NBS SRM 1632a)

	µg Zn/g	µg Cd/g	µg Pb/g
	26.932	0.16895	12.206
	26.938	0.16871	12.203
	26.973	0.16896	12.205
	26.919	0.16869	12.206
	26.914	0.16893	12.204
Average	26.935	0.16885	12.205
Std. Dev.	0.023	0.00014	0.001
Range	0.059	0.00027	0.003

As mentioned previously, the precision of the isotope dilution method allows the identification of sample inhomogeneities which are often masked by the imprecision of other analytical techniques. The data in Table III represent 15 separate mass spectrometric analyses on a one g sample taken from bottle No. 1 of SRM 1632a. These data are typical of the precision expected from the mass spectrometric measurements. Comparison of the data in Table III with those in Table II indicates that the variation between bottles is the result of sample inhomogeneity rather than measurement imprecision. Statements on the accuracy of the concentration measurements must include not only the measurement precision and blank uncertainty contribution, but also components to cover possible sources of systematic errors such as the uncertainty in the concentration of the separated isotope and the isotopic composition of the separated isotope and sample. Thus, although the mass spectrometric precision for Pb analyses is generally less than 0.1%, an accuracy statement of $\pm$ 0.2% is generally assigned to the data. At present the accuracy of the Zn and Cd analyses is estimated to be $\pm$ 0.5%. This value is expected to decrease as experience is gained and sources of possible systematic errors are further investigated.

CONCLUSIONS

Isotope dilution mass spectrometry is an accurate and precise analytical technique capable of determining Zn, Cd and Pb concentrations from the % level into the ng/g concentration range where the analytical blank becomes a limiting factor in the accuracy. The technique is particularly useful for the standardization of reference materials and for measuring small sample inhomogeneities.

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